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Carboxylate-Based Receptors for the Recognition of Carbohydrates in Organic and Aqueous Media

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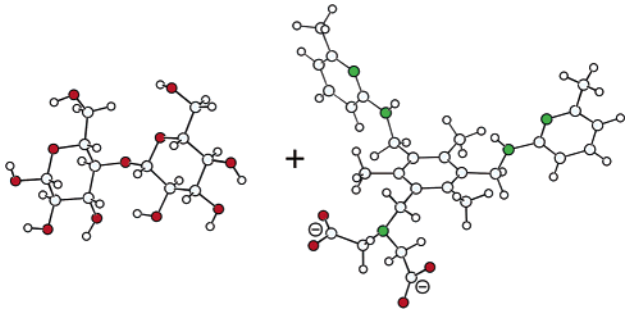

Acyclic receptors containing neutral and ionic hydrogen-bonding sites, such as amino-pyridine and carboxylate groups, were prepared and their binding properties toward neutral sugar molecules were studied. The binding studies with disodium and bis(tetramethylammonium) salts containing the dianion **11** have revealed that this type of receptor molecule is able to recognize the selected sugars in both organic and aqueous media. The carboxylate/pyridine-based receptor **11** exhibits in chloroform at least a 100-fold higher affinity for glucopyranosides than the previously described triarmed pyridine-based receptor **1**, incorporating only neutral hydrogen-bonding sites. A substantial drop in the association constants is expectedly observed for an ester analogue of **11**, compound **9**. The dicarboxylate **11** is able to form complexes in water with methyl β-D-glucopyranoside and D-cellobiose, with a preference for the disaccharide. The studies show the importance of charge-reinforced hydrogen bonds in the recognition of carbohydrates.

Introduction

Artificial carbohydrate receptors provide valuable model systems to study the basic molecular features of carbohydrate recognition. On the other hand, the binding motifs observed in the available crystal structures of protein-carbohydrate complexes[1] inspire the development of artificial receptor molecules.[2,3] Understanding how carbohydrates interact with their protein receptors is of particular importance due to the key roles that sugar molecules play in a variety of biological processes.[4] Advances in this area provide useful information for the design of both protein and carbohydrate mimetics,[5] which may serve as a basis for the development of saccharide sensors[6] or therapeutics that intervene in biologically important carbohydrate recognitions.[5,7]

X-ray analyses of the protein-carbohydrate complexes have revealed that the sugar-binding sides are almost completely populated by residues with planar polar side chains with at least two functional groups capable of engaging in cooperative and bidentate hydrogen bonds.[1] The hydrogen bonds have both the neutral and the ionic character, as shown in Scheme 1. Carboxylate side chains play a role in anomeric and epimeric specific sugar recognition. The carboxylate group is able to form bidentate hydrogen bonds to vicinal hydroxyl groups, as illustrated in Scheme 1a,b. In the crystal structure of peanut agglutinin with bound disaccharide Gal(β1−3)GalNAc, for example, the carboxy group of the aspartic acid side chain makes

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(1) (a) Quiocho, F. A. *Pure. Appl. Chem.* **1989**, *61*, 1293−1306. (b) Quiocho, F. A.; Wilson, D. K. *Nature* **1989**, *340*, 404−408. (c) Vyas, N. K.; Vyas, M. N.; Quiocho, F. A. *Science* **1988**, *242*, 1290−1295. (d) Weiss, W. I.; Drickamer, K. *Annu. Rev. Biochem.* **1996**, *65*, 441−473. (e) Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347−374. (f) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637−674. (g) Ravishankar, R.; Ravindran, N.; Suguna, A.; Surolia, A.; Vijayan, M. *Curr. Sci.* **1997**, *72*, 855. (h) Spurlino, S. P.; Lu, G.-Y.; Quiocho, F. A. *J. Biol. Chem.* **1991**, *266*, 5202−5219.

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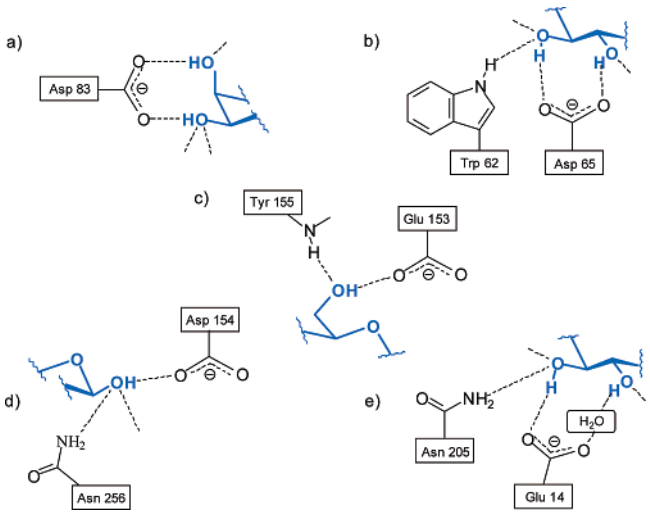
SCHEME 1. Common Hydrogen-Bonding Arrangements for Carboxylate Groups in the Crystal Structures of Protein−Carbohydrate Complexes[a]



[a] Examples of hydrogen bonds in the complexes of peanut agglutinin with Gal(β1−3)GalNAc[1e,g] (a), maltose-binding protein with maltose[1h] (b,c), D-galactose-binding protein with D-glucose[1a,c] (d), and L-arabinose-binding protein with L-arabinose[1a] (e).

hydrogen bonds with the 3- and 4-hydroxy groups of the galactose unit (Scheme 1a).[1e,g] Bidentate hydrogen bonds with the 2- and 3-hydroxy groups can be observed in the crystal structure of the complex between the maltose binding protein (MBP) and maltose (Scheme 1b).[1h] Water molecules are often involved in protein−carbohydrate interations, mediating hydrogen bonds between residues and sugar. In the complex of arabinose binding protein with L-arabinose, for example, the water molecule is observed to hydrogen bond to 2-OH of the bound sugar and the oxygen atom of Glu14[1a] (Scheme 1e).

The crystal structure of the MBP−maltose complex provides a particularly interesting example of the extensive use of polar and aromatic residues in binding sugar molecules. Quiocho and co-workers have established that, with the exception of two NH groups (one from the peptide NH of Tyr[155] and the other from the Trp[62] side chain; see Scheme 1b,c), all of the side chains that form direct hydrogen bonds with the maltose are charged, four carboxylate, one quanidinium, and one ammonium groups. Thus, a large number of the direct hydrogen bonds (9 out of 11) in the MBP−maltose complex are between neutral sugar-OH groups and charged residues.[1h]

The potential of anionic centers for carbohydrate recognition has been explored by several groups. Particular attention has been paid to phosphates and phoshonates.[3a,8] The development of anionic mono- and bisphosphonate derivatives that bind alkyl glucosides strongly in polar organic solvents, such as CD_3CN, has been reported by Hamilton and Das.[8a,b] Diederich and Neidlein have synthesized an optically active, 1,1′-binaphthyl-derived cyclophane receptor with a preorganized central cavity lined with four anionic phosphodiester groups for ionic hydrogen bonds.[8c] Calix[4]arene-based receptors, in which a charged phosphate group cooperates with a peptide hydrogen-bonding donor group and acceptor groups in the binding process, have been studied by Ungaro and co-workers.[2l]

Schneider et al. have explored systematically different anionic functions for the complexation of aliphatic hydroxyl compounds, including carbohydrates, in chloroform. The anions studied comprise carboxylates, phosphates, sulfonates, and halides (peralkylammonium salts containing different anions, for example, hexadecyltrimethylammonium benzoate, have been used). The aim of this study was to provide data characterizing the acceptor qualities of the anions, particularly in combination with hydroxyl groups as hydrogen-bond donors.[8d]

A fused-pyridine host, containing two carboxylate groups, has been shown to bind cationic monosaccharides such as D-glucosamine·HCl, D-galactosamine·HCl, and D-mannosamine·HCl with high affinity in methanol.[8e]

Carbohydrate recognition in aqueous solution through noncovalent interactions remains an important challenge in artificial receptor chemistry (particularly, the neutral carbohydrates are challenging substrates to recognize).[2a,9] Davis et al. have shown that a polyanion receptor with a tricyclic core is able to bind carbohydrates in water with low affinities, but significant selectivities.[2a] The tricyclic architecture was designed to provide both apolar and polar contacts to a saccharide. Cyclophane systems derived from two cryptand and two porphyrin units were synthesized by Schmidtchen and co-workers. These receptors are able to bind saccharides in highly competitive

(2) For some recent examples of artificial carbohydrate receptors using noncovalent interactions, see: (a) Klein, E.; Crump, M. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2005**, *44*, 298−302. (b) Mazik, M.; Cavga, H.; Jones, P. G. *J. Am. Chem. Soc.* **2005**, *127*, 9045−9052. (c) Mazik, M.; Radunz, W.; Boese, R. *J. Org. Chem.* **2004**, *69*, 7448−7462. (d) Mazik, M.; Sicking, W. *Tetrahedron Lett.* **2004**, *45*, 3117−3121. (e) Benito, J. M.; Meldal, M. *QSAR Comb. Sci.* **2004**, *23*, 117−129. (f) Vacca, A.; Nativi, C.; Cacciarini, M.; Pergoli, R.; Roelens, S. *J. Am. Chem. Soc.* **2004**, *126*, 16456−16464. (g) Gupta, G.; Lowe, C. R. *J. Mol. Recognit.* **2004**, *17*, 218−235. (h) Velasco, T.; Lecollinet, G.; Ryan, T.; Davis, A. P. *Org. Biomol. Chem.* **2004**, *2*, 645−647. (i) Fang, J.-M.; Selvi, S.; Liao, J.-H.; Slanina, Z.; Chen, C.-T.; Chou, P.-T. *J. Am. Chem. Soc.* **2004**, *2*, 645−647. (j) Welti, R.; Abel, Y.; Gramlich, V.; Diederich, F. *Helv. Chim. Acta* **2003**, *86*, 548−562. (k) Wada, K.; Mizutani, T.; Kitagawa, S. *J. Org. Chem.* **2003**, *68*, 5123−5131. (l) Segura, M.; Bricoli, B.; Casnati, A.; Muñoz, E. M.; Sansone, F.; Ungaro, R.; Vicent, C. *J. Org. Chem.* **2003**, *68*, 6296−6303. (m) Welti, R.; Diederich, F. *Helv. Chim. Acta* **2003**, *86*, 494−503. (n) Dukh, M.; Saman, D.; Lang, K.; Pouzar, V.; Cerny, I.; Drasar, P.; Král, V. *Org. Biomol. Chem.* **2003**, *1*, 3458−3463. (o) Ishi-I, T.; Mateos-Timoneda, M. A.; Timmerman, P.; Crego-Calama, M.; Reinhoudt, D. N.; Shinkai, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 2300−2305.

(3) For reviews, see: (a) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2979−2996. (b) Hartley, J. H.; James, T. D.; Ward, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155−3184. (c) Armitage, B. A. *Ann. Rep. Prog. Chem. Sect. B: Org. Chem.* **2002**, *98*, 503−541 (Bioinspired organic chemistry). (d) Striegler, S. *Curr. Org. Chem.* **2003**, *7*, 81−102.

(4) (a) Osborn, H.; Khan, T. *Oligosaccharides. Their synthesis and biological roles*; Oxford University Press: New York, 2000. (b) Sears, P.; Wong, C.-H. *Cell. Mol. Life Sci.* **1998**, *54*, 223. (c) Varki, A. *Glycobiology* **1993**, *3*, 97−130. (d) Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1−27.

(5) For a review on carbohydrate mimetics, see: Sears, P.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2300−2324

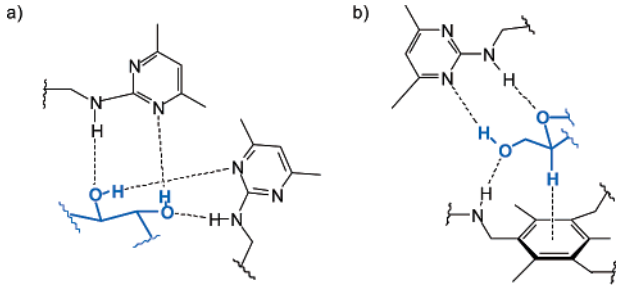
(6) (a) For a review on boronic acid-based carbohydrate sensors using covalent interactions for sugar binding, see: Shinkai, S.; James, T. D. *Top. Curr. Chem.* **2002**, *218*, 159−200. (b) For a functional assay for heparin in serum using a boronic acid-based receptor, see: Wright, A. T.; Zhong, Z.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2005**, *44*, 5679−5682.

(7) As suggested by Davis et al. in ref 3a, "synthetic carbohydrate receptors could be used as drugs (e.g., anti-infective agents) to target cell types (acting as synthetic antibodies) and to transport saccharides or related pharmaceuticals across cell membranes".

(8) (a) Das, G.; Hamilton, A. D. *J. Am. Chem. Soc.* **1994**, *116*, 11139−11140. (b) Das, G.; Hamilton, A. D. *Tetrahedron Lett.* **1997**, *38*, 3675−3678. (c) Neidlein, U.; Diederich, F. *Chem. Commun.* **1996**, 1493−1494. (d) Coterón, J. M.; Hacket, F.; Schneider, H.-J. *J. Org. Chem.* **1996**, *61*, 1429−1435. (e) Tamaru, S.; Yamamoto, M.; Shinkai, S.; Khasanov, A. B.; Bell, T. W. *Chem.−Eur. J.* **2001**, *7*, 5270−5276.

(9) (a) Král, V.; Rusin, O.; Schmidtchen, F. P. *Org. Lett.* **2001**, *3*, 873−876. (b) Hubbard, R. D.; Horner, S. R.; Miller, B. L. *J. Am. Chem. Soc.* **2001**, *123*, 5810−5811. (c) Yanagihara, R.; Aoyama, Y. *Tetrahedron Lett.* **1994**, *35*, 9725−9728.

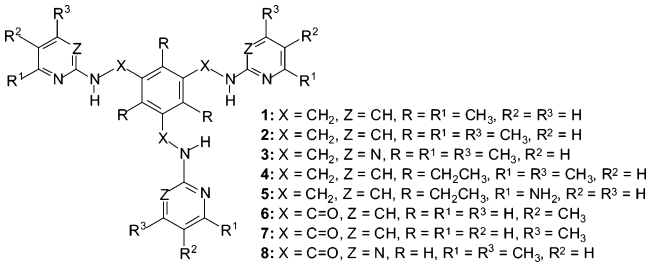
SCHEME 2. Examples of Hydrogen-Bonding Motifs Observed in the Crystal Structure of the Complex between Receptor 3 and Octyl β-D-Glucopyranoside



media (in water/methanol) with a preference for trisaccharides.[9a] Miller and co-workers have employed substituted *tert*-cyclopentanes as receptors for the carbohydrate unit of lipid A in aqueous solution.[9b] Aoyama and Yanagihara have used strongly anionic macrocyclic hosts for the recognition of carbohydrates in water.[9c]

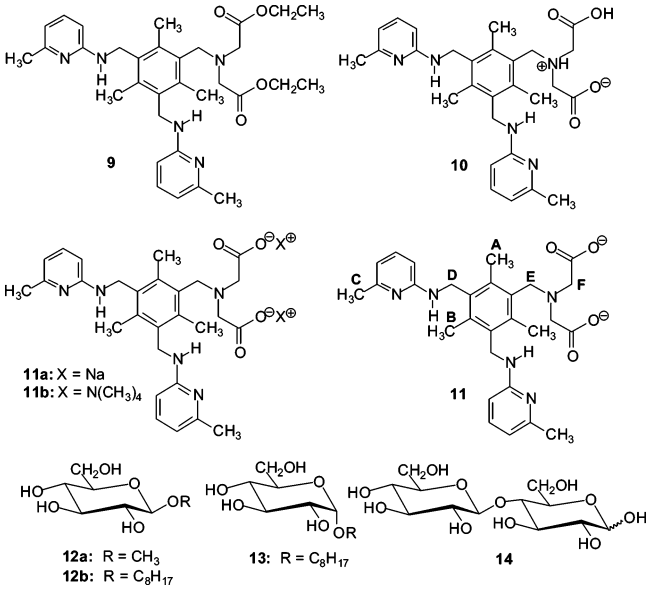
Our interest in this area concentrates on carbohydrate receptors that possess a relatively simple, acyclic structure and that are expected to complex carbohydrates through neutral and ionic hydrogen bonds in combination with the interactions between the faces of the sugar and the aromatic rings of the receptor.

Recently, we have reported that receptors of types **1**−**8** perform effective recognition of carbohydrates through multiple interactions.[2b,c,d,10] The possible binding modes were discussed in detail on the basis of chemical shift changes in ^{1}H NMR spectra and molecular modeling calculations.[2c,d,10] In addition, the suggested binding modes were also supported by X-ray analyses of the complexes formed between the acyclic receptor system and the sugar molecules.[2b] Noteworthy, the crystal structures of the complexes of receptors **2** and **3** with methyl or octyl β-D-glucopyranoside have proved to contain many of the molecular features associated with protein−carbohydrate interactions. In these complexes, all OH groups and the ring oxygen atom of the bound glucopyranoside are involved in the formation of hydrogen bonds (including cooperative and bidentate hydrogen bonds; for examples, see Scheme 2), whereas the CHs of the sugar molecule participate in the formation of the CH···π interactions with the central phenyl ring of the receptor **2** or **3** (Scheme 2b).[2b]



In this paper, we describe the synthesis and binding properties of new acyclic receptors (Scheme 3) containing both neutral and ionic hydrogen-bonding sites, such as amino-pyridine[11] and carboxylate groups as the recognition units used in nature. As in previously described artificial systems,[2b] the participation of

SCHEME 3 Structures of Receptors and Sugars Studied



the phenyl ring of the receptor in the interactions with sugar C−H bonds is expected to provide additional stabilization of the receptor−sugar complexes (particularly in water, the CH···π interactions should be reinforced by the hydrophobic effect).

Results and Discussion

The synthesis of the disodium and bis(tetramethylammonium) salts of the dicarboxylate receptor **11** (**11a** and **11b**, respectively) started from 1,3,5-tris(bromomethyl)-2,4,6-trimethyl-benzene (**15**),[12] which was converted into the diester **9** via a reaction with 1 equiv of iminodiacetic acid diethyl ester, followed by the reaction with 2 equiv of 2-amino-6-methylpyridine. The separation of the product **9** and the byproducts **1** and **16** was carried out by column chromatography (see Experimental Section). The crystal structure of **9** is shown in Figure 1. The hydrolysis of **9** gave the sodium salt **11a** and the diacid **10**, which was converted into the tetramethylammonium salt **11b**, as shown in Scheme 4.

Complexations of sugars with the disodium salt **11a** were measured in pure water, while the use of tetramethylammonium ions as counterions in **11b** allowed the measurements in both chloroform and water solutions. In addition, the recognition properties of the diester **9** were tested in chloroform solutions. The interactions of the receptors and saccharides in organic and aqueous media were investigated by ^{1}H NMR binding titrations. Methyl and octyl-β-D-glucopyranosides (**12a** and **12b**), octyl α-D-glucopyranoside (**13**), as well as D-cellobiose (**14**) were selected as substrates for the receptors.

Although molecular recognition of carbohydrates is relevant in water, the complexation studies in organic media are also of high importance. Quiocho et al. have shown that the hydrogen bonds between sugar-binding proteins and essential recognition determinants on sugars are shielded from bulk solvent, meaning

(10) (a) Mazik, M.; Bandmann, H.; Sicking, W. *Angew. Chem., Int. Ed.* **2000**, *39*, 551−554. (b) Mazik, M.; Sicking, W. *Chem.—Eur. J.* **2001**, *7*, 664−670. (c) Mazik, M.; Radunz, W.; Sicking, W. *Org. Lett.* **2002**, *4*, 4579−4582.

(11) As noted by Anslyn et al., the 2-aminopyridine unit can be regarded as a heterocyclic analogue of the asparagine/glutamine primary amide side chain, see: Huang, C.-Y.; Cabell, L. A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 2778−2792.

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

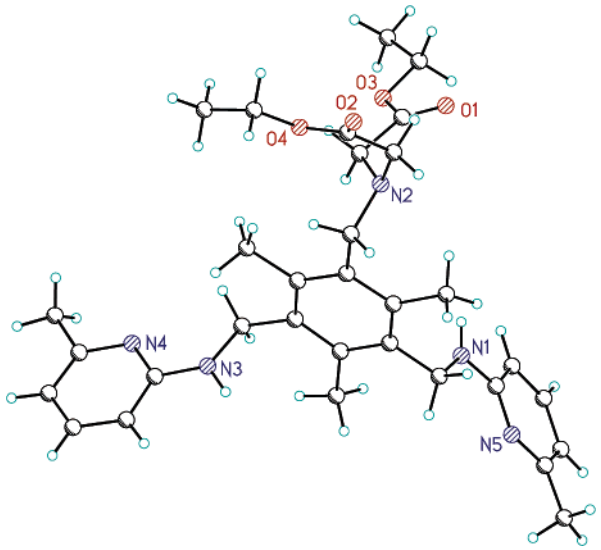
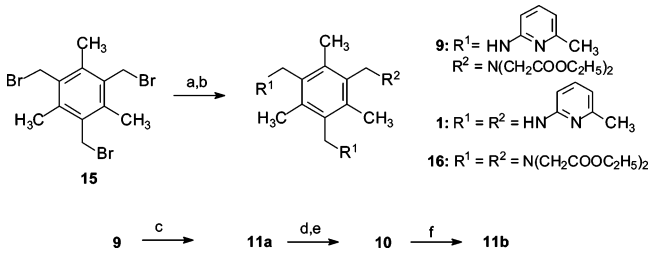


FIGURE 1. Crystal structure of **9**.

SCHEME 4 [a]



[a] Key: (a) 1 equiv of $HN(CH_2COOCH_2CH_3)_2$, K_2CO_3, CH_3CN, 25 °C, 45 min; (b) 2 equiv of 2-amino-6-methylpyridine, K_2CO_3, CH_3CN, 25 °C, 48 h; yield of **9** = 33%; (c) THF, MeOH, aqueous NaOH; (d) 5% HCl; (e) NEt_3, $CHCl_3$; yield = 93%; (f) 2 equiv of $HON(CH_3)_4$, MeOH; yield = 100%.

that they exist in a lower dielectric environment.[1a,b,c,h] Thus, the recognition of sugars in lipophilic solvents provides important information about the factors that contribute to the affinity between receptors and saccharides and offers an important screen for effective recognition motifs for carbohydrates.

Binding Studies in Organic Media. Titrations in $CDCl_3$ with receptors **9** and **11b** were run at constant receptor concentration. Dilution experiments with **11b** showed that self-association at concentrations below 0.9 mmol/l was negligible.

The complexation between the receptor **11b** and pyranosides **12b** and **13** was evidenced by several changes in the NMR spectra. During the titration of **11b** with β-glucopyranoside **12b** the signal due to the amine NH moved downfield by about 1.6 ppm (see Figure 2); the addition of 2 equiv of sugar led to practically the complete complexation of **11b**. Furthermore, the ^{1}H NMR spectra showed some changes in the chemical shifts of the CH_2 (protons D, E, and F; for labeling, see Scheme 3) and CH_3 resonances (protons A, B, and C), as well as the pyridine C*H* protons (see Supporting Information, Figure S1). The ^{1}H NMR signals of the NHCH_2 protons (protons D) moved upfield by 0.15 ppm with strong broadening (see Figure 3). The E resonances broaden during the titration and were almost unobservable after the addition of only 0.2 equiv of glucopyranoside **12b**, and the signals of the protons F were overlapping

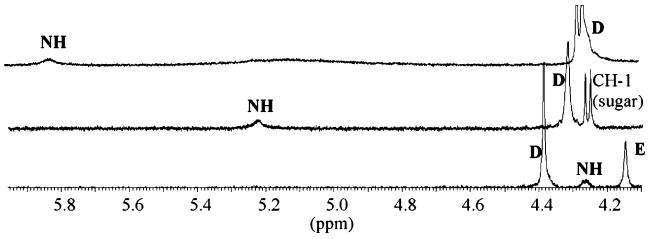


FIGURE 2. Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$) of **11b** after the addition of (from bottom to top) 0, 0.86, and 2.96 equiv of **12b** ([**11b**] = 0.85 mM).

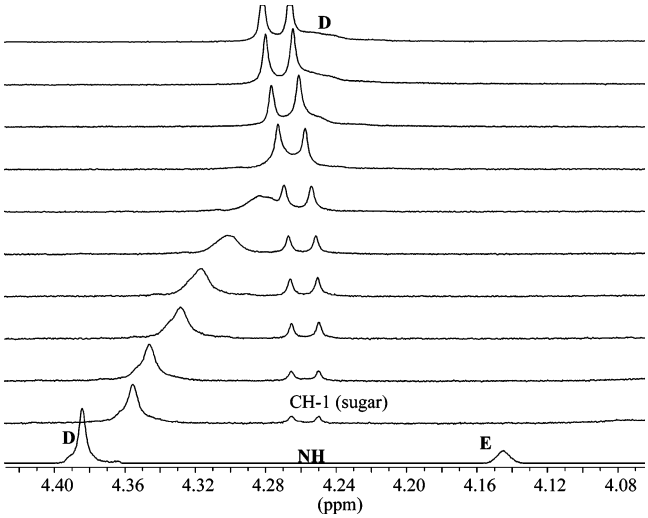


FIGURE 3. Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$) of **11b** after the addition of (from bottom to top) 0, 0.34, 0.51, 0.69, 0.86, 1.21, 1.55, 2.16, 2.96, 3.89, and 4.32 equiv of **12b** ([**11b**] = 0.85 mM).

during the titration. The CH_3 signals moved only in the range 0.03−0.05 ppm.

The NH signals were monitored for the determination of the binding constants; the typical titration curve is shown in Figure 4a. Both the curve fitting of the titration data[13] and the mole ratio plots[14] suggested the existence of 1:1 and 1:2 receptor/sugar complexes in the chloroform solution, similar to the previously described triarmed pyridine-based receptor **1**. The binding constants for **11b**·**12b** were found to be 119 420 M^{-1} (K_{a1}) and 4730 (K_{a2}). These results clearly show that the interactions with the carboxylate groups of **11b** play a significant role and influence considerably the receptor affinity (for comparison of the binding constants for **11b**·**12b** and **1**·**12b**, see Table 1).

The ^{1}H NMR titrations of **11b** with α-glucopyranoside **13** produced similar spectral changes. Particularly, the signal due to the amine NH of **11b** moved substantially downfield ($\Delta\delta_{max}$ = 1.60 ppm). In addition, changes in the chemical shifts of the methylene (see, for example, Figure S2), methyl, and pyridine protons were also established. The fit of NMR shift changes of the NH of **11b** during the titration with **13** agreed again with the mixed 1:1 and 1:2 receptor/sugar binding model

(13) The titration data were analyzed by nonlinear regression analysis, using the Hostest program: Wilcox, C. S.; Glagovich, N. M. *HOSTEST 5.6*; University of Pittsburgh: Pittsburgh, PA, 1994.

(14) Tsukube, H.; Furuta, H.; Odani, A.; Takeda, Y.; Kudo, Y.; Inoue, Y.; Liu, Y.; Sakamoto, H.; Kimura, K. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davis, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon: Oxford, U.K., 1996; Vol. 8, p 425.

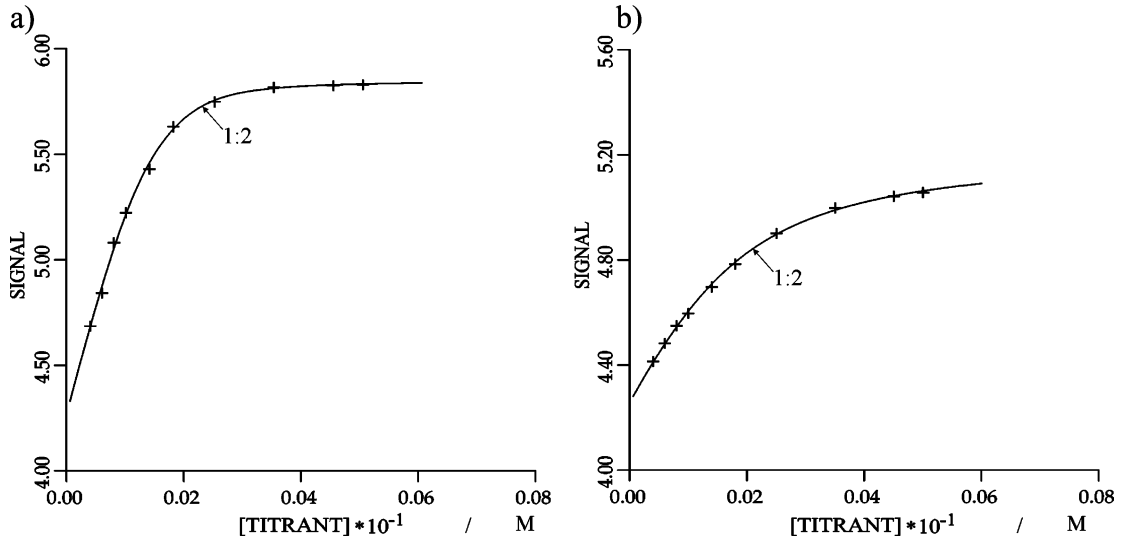


FIGURE 4. Plot of the observed (×) and calculated (—) downfield chemical shifts of the NH resonances of **11b** (a) and **9** (b) as a function of added glucopyranoside **12b**. (a) [**11b**] = 0.85 mM; equiv of **12b** = 0.34, 0.51, 0.69, 0.86, 1.21, 1.55, 2.16, 3.03, 3.89, and 4.32. (b) [**9**] = 0.93 mM; equiv of **12b** = 0.43, 0.64, 0.86, 1.07, 1.50, 1.89, 2.60, 3.77, 4.84, and 5.38.

TABLE 1. Association Constants K_a (M^{-1}) for Receptors 11, 9, and 1 and Sugars 12a, 12b, 13, and 14

host−guest complex	solvent	K_{a1}	K_{a2}[c]	$\Delta\delta_{max}$[d] ($\Delta\delta_{obs}$)[e] [ppm]
11b·12b	$CDCl_3$[a]	119 420	4730	NH: 1.60 (1.60)[f]
11b·13	$CDCl_3$[a]	21 500	3900	NH: 1.62 (1.59)[f]
11a·12a	H_2O/D_2O[b]	2	72	CH: −0.04(−0.03)[g]
11a·14	H_2O/D_2O[b]	305	66	CH: 0.03 (0.03)[h]
9·12b	$CDCl_3$[a]	5850	720	NH: 0.96 (0.80)[f]
9·13	$CDCl_3$[a]	540	150	NH: 1.08 (0.55)[f]
1·12b[i]	$CDCl_3$[a]	10 500	250	NH: 1.30 (1.22)[f]
1·13[i]	$CDCl_3$[a]	690		NH: 1.42 (1.07)[f]

[a] $CDCl_3$ was stored over activated molecular sieves and deacidified with Al_2O_3. For each system, at least three titrations were carried out. The error in a single K_a estimation was <10%. [b] H_2O/D_2O, 93:7, v/v. [c] Receptor/sugar complex, 1:2. [d] Change in chemical shift at saturation binding, values provided by HOSTEST.[13] [e] Largest change in chemical shift observed during the titration. [f] Complexation-induced shifts observed for the amine-NH of the receptor (the concentration of the receptor was kept constant and that of the sugar was varied). [g] Upfield complexation-induced shifts observed for the protons A of the receptor. [h] Downfield complexation-induced shifts observed for the protons C of the receptor. [i] Results from ref 10c.

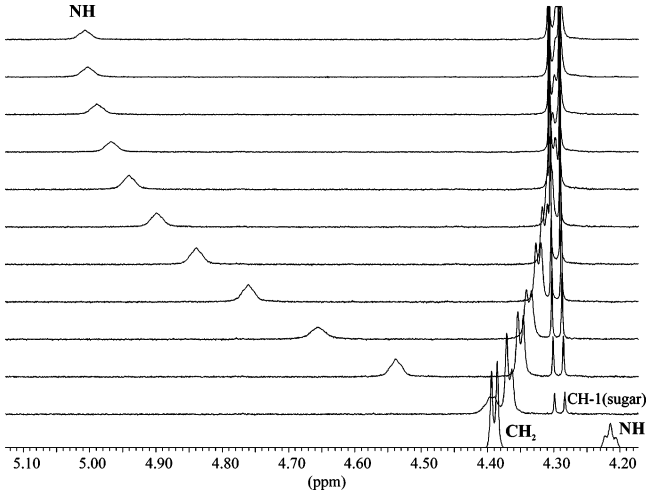


FIGURE 5. ^1H NMR titration of receptor **9** with β-glucopyranoside **12b**. ^1H NMR spectra ($CDCl_3$) of **9** after the addition of (from bottom to top) 0, 0.55, 1.11, 1.67, 2.23, 2.79, 3.35, 3.91, 4.47, 5.03, 5.59, and 6.00 equiv of **12b** ([**9**] = 0.90 mM).

(Figure S2a). The association constants of 21 500 (K_{a1}) and 3900 M^{-1} (K_{a2}) were determined. Thus, similar to **1**, the dicarboxylate **11** shows a preference for the β-anomer in the recognition of glucopyranosides. It should be noted that, with the exception of the amidopyridine receptors **6** and **7**,[2c,d] which are sterically less hindered at nitrogen, all the previously described pyridine- and pyrimidine-based receptors bind the β-glucopyranoside better than the α-anomer (these results are, among other things, connected with the tendency for the formation of different intramolecular hydrogen bonds in the two anomers).[15]

The comparison of the overall binding constants β_2 indicates that the dicarboxylate **11** exhibits at least 100-fold higher affinity for β-glucopyranoside **12b**, than the previously described triarmed pyridine-based receptor **1**, incorporating only neutral hydrogen-bonding sites. The distinction between the affinities toward the α-glucopyranoside **13** is even higher. These results demonstrate the importance of ionic hydrogen bonds in the molecular recognition of carbohydrates.

The studies in the area of drug design show with particular clarity the importance of charge-reinforced hydrogen bonds in receptor−substrate interactions.[16] These studies found that a neutral−neutral hydrogen bond is worth up to about 1.5 kcal mol^{-1}, which is equivalent to a maximum 15-fold increase in binding, whereas a hydrogen bond between a charged and a neutral component can contribute up to 3000-fold to the binding

(15) . The axial 1-alkoxy group in the α-anomer can form intramolecular hydrogen bonds with the 2-OH group more easily than the equatorial 1-alkoxy substituent in the β-anomer. Thus, the 2-OH in β-glucopyranoside is relatively free from intramolecular hydrogen bonding and can interact with a receptor molecule more strongly.

(16) (a) Davis, A. M.; Teague, S. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 736−749. (b) Fersht, A. R.; Shi, J.-P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M. Y.; Winter, G. *Nature* **1985**, *314*, 845−851. (c) Williams, D. H.; Searle, M. S.; Mackay, J. P.; Gerhard, U.; Maplestone, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1172−1178.

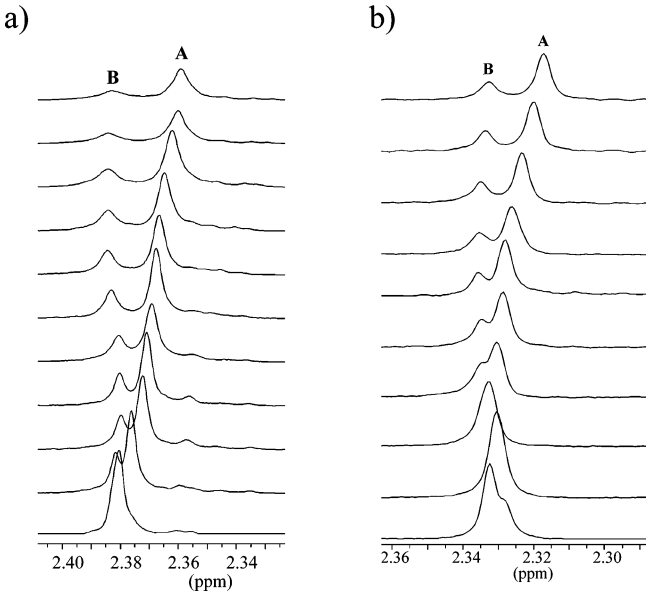


FIGURE 6. (a) Partial ^{1}H NMR spectra of **11b** in $CDCl_3$ (CH_3 resonances, A and B, are shown) after the addition of (from bottom to top) 0, 0.31, 0.47, 0.63, 0.79, 1.10, 1.42, 1.98, 2.77, 3.56, and 3.96 equiv of **13** ([**11b**] = 0.85 mM). (b) Partial ^{1}H NMR spectra of **11a** in H_2O/D_2O (93:7, v/v) after the addition of (from bottom to top) 0, 29, 44, 58, 87, 116, 145, 174, 233, 291, and 349 equiv of **14** ([**11a**] = 0.72 mM).

of a substrate (up to 4.7 kcal mol^{-1}). Hydrophobic interactions contribute a minimum of a 3.2-fold increase in binding per methyl group. They play a major role in the affinity of most drugs for their receptors.[16a]

The complexation between the diester **9** and the glucopyranosides **12b** and **13** was also evidenced by the significant downfield shift of the receptor amine protons (see Figure 5 and Figure S3), as well as changes in the chemical shifts of C*H*$_2$, C*H*$_3$, and pyridine C*H* resonances**.** The curve fitting of the titration data for receptor **9** and both sugars (see, for example, Figure 4b) suggested again the existence of both 1:1 and 1:2 receptor/sugar complexes in the chloroform solutions, with a stronger association constant for the 1:1 binding and a weaker association constant for the 1:2 receptor/sugar complex. The association constants of 5850 (K_{a1}) and 720 M^{-1} (K_{a2}) were determined for **9·12b** (Table 1), whereas the binding constants for **9·13** amount to 540 (K_{a1}) and 150 M^{-1} (K_{a2}). Thus, the complexes formed between the glucopyranosides and the diester **9** are expectedly much less stable than those formed with the dicarboxylate **11**.

Binding Studies in Aqueous Media. Following the success with **11b** in organic media, we investigated the properties of the dicarboxylate **11** in water. The ^{1}H NMR titration experiments were carried out by adding increasing amounts of the corresponding sugar to a solution of the salt **11a** or **11b**. To compare the binding properties of our acyclic receptor **11** with the recently described tricyclic polyamide receptor, we chose the conditions used by Davis and co-workers.[2a] The complexations were carried out in D_2O and H_2O/D_2O (93:7, v/v) mixtures, using methyl β-D-glucopyranoside (**12a**) and D-cellobiose (**14**) as probes. The addition of sugars **12a** and **14** into the aqueous solutions of the salts **11a** and **11b** yielded qualitatively similar NMR effects.

Dilution experiments with **11a** showed that self-association in water is negligible at concentrations below 0.90 mmol/L, which was above the concentration used in NMR titrations. The receptor NH protons, which are directly involved in the binding site and show large downfield shifting upon complexation with sugars in $CDCl_3$, are under the chosen conditions unobservable. Consequently, complexation-induced chemical shifts of the receptor CH units were monitored. The ^{1}H NMR titrations with glucopyranoside **12a** or disaccharide **14** produced chemical shift changes of C*H*$_2$, C*H*$_3$, as well as pyridine C*H* groups; however, the complexation-induced shifts were small (in the range of 0.02−0.05 ppm). For the determination of the binding constants, the CH_3 signals (protons A and C; for labeling, see Scheme 3) were monitored (CH_2 signals were partially overlapping during the titrations). Similar shift changes of the CH_3 groups were also observed in $CDCl_3$ during the titration of both **11b** and **9** with glucopyranosides **12b** and **13**, however, already at lower

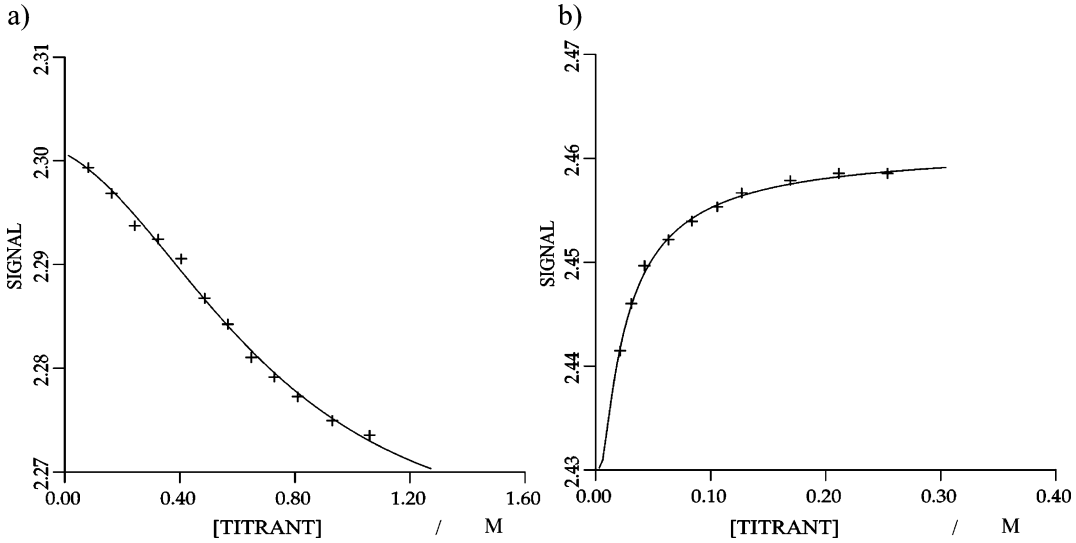


FIGURE 7. ^{1}H NMR titration of receptor **11a** with sugars **12a** and **14** in H_2O/D_2O (93:7, v/v). (a) Upfield chemical shifts of the protons A of **11a** are plotted against increasing β-D-glucopyranoside (**12a)** concentration; [**11a**] = 0.81 mM; equiv of **12a** = 70, 140, 215, 287, 358, 430, 502, 574, 645, 717, 820, and 900. (b) Downfield chemical shifts of the protons C of **11a** are plotted against increasing D-cellobiose (**14**) concentration; [**11a**] = 0.72 mM; equiv of **14** = 29, 44, 58, 87, 116, 145, 174, 233, 291, and 349.

receptor/sugar ratios (for comparison, see Figure 6). The determination of binding constants on the basis of such small complexation-induced shifts may be associated with a large error. In such cases, careful control of the experimental conditions is of particular importance. For each system (**11a·12a** and **11a·14**), five titration experiments were carried out, three in H_2O/D_2O (93:7, v/v) and two in D_2O. During the titrations of **11a** with glucopyranoside **12a**, the protons A of **11a** shifted upfield and the protons C almost did not move (<0.01 ppm).

The addition of cellobiose (**14**), that had been equilibrated overnight, caused shifts of both A and C protons (upfield and downfield shifts, respectively). In contrast to the titrations with cellobiose (see Figure 7b), the receptor shifts observed during the titration with glucopyranoside **12a** varied only in a nearly linear way with the sugar concentration (Figure 7a), indicating a weaker complex. The curve fitting of all the titration data suggested the existence of both 1:1 and 1:2 receptor/sugar complexes in water. The K_a values for **11a·12a** were determined to be 2 (K_{a1}) and 72 M^{-1} (K_{a2}; Table 1), indicating the formation of the stronger 1:2 receptor−monosaccharide complexes in water. In contrast, the analysis of the titration data obtained with cellobiose indicated stronger 1:1 binding, followed by a weaker association of the second sugar molecule, as the binding constants were found to be 305 (K_{a1}) and 66 M^{-1} (K_{a2}). Thus, the acyclic receptor recognized in water both glucopyranoside and cellobiose, with a preference for the disaccharide.

Conclusion

The receptors studied comprise carboxylates as the entities used in nature, as well as 2-aminopyridine groups as heterocyclic analogues of the asparagine/glutamine side chains. These recognition units are interconnected by a phenyl spacer, which was incorporated into the receptor structure to provide additional apolar contacts to a saccharide (similar to sugar-binding proteins, which commonly place aromatic surfaces against patches of sugar CH groups). The binding studies with disodium and bis-(tetramethylammonium) salts of the dicarboxylate **11** (**11a** and **11b**, respectively) showed that the incorporation of suitably positioned carboxylate groups into the receptor structure enhances the binding considerably. The acyclic receptor is able to form complexes with neutral sugar molecules both in organic and in aqueous media. In chloroform, receptor **11** exhibits at least a 100-fold higher affinity for glucopyranosides than the previously described triarmed pyridine-based receptor **1**, incorporating only neutral hydrogen-bonding sites. Similar to **1**, the dicarboxylate **11** shows a preference for the β-anomer in the recognition of glucopyranosides. Furthermore, the complexes formed between the receptor **11** and glucopyranosides are expectedly much more stable than those formed with the neutral ester/pyridine-based receptor **9**.

The dicarboxylate **11** is able to form weak complexes in water with methyl β-D-glucopyranoside and D-cellobiose. The spectral changes observed in the titrations with the cellobiose **14** were more substantial than those seen in the titrations with the glucopyranoside **12a**, indicating stronger binding between the disaccharide and the receptor **11**. As in natural receptors, the combination of neutral and ionic hydrogen bonds, as well as hydrophobic interactions, provides impetus for the binding of sugars in water. The formation of charge-reinforced hydrogen bonds between the carboxylate groups of **11** and the hydroxy groups of sugar molecules provides the major driving force for the complexation of neutral sugars in aqueous solutions.

This type of receptor provides an opportunity to study carbohydrate recognition both in organic and in aqueous solutions. The simple acyclic structure offers the possibility of an easy variation of the receptor structure to modulate the binding properties of the receptor molecules.

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Supporting Information Available: Syntheses of compounds **9**, **10**, **11a**, and **11b**. X-ray data for compound **9**. ^1H and ^{13}C NMR spectra of compounds **9**, **10**, **11a**, and **11b**. Examples of ^1H NMR titrations of receptors **9**, **11a**, and **11b** with carbohydrates **12**−**14** in $CDCl_3$ and water. This material is available free of charge via the Internet at http://pubs.acs.org.

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