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Isopropylamino and Isobutylamino Groups as Recognition Sites for Carbohydrates: Acyclic Receptors with Enhanced Binding Affinity toward β-Galactosides

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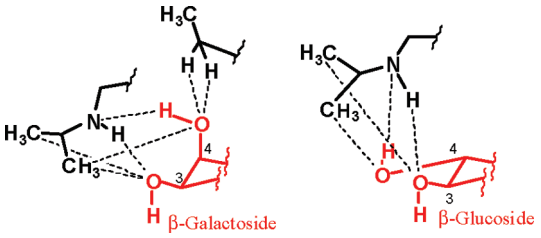

Binding motifs observed in the crystal structures of protein−carbohydrate complexes, in particular the participation of the isopropyl/isobutyl side chain of valine/leucine in the formation of van der Waals contacts, have inspired the design of new artificial carbohydrate receptors. The new compounds, containing a trisubstituted triethylbenzene core, were expected to recognize sugar molecules through a combination of NH···O and OH···N hydrogen bonds, CH···π interactions, and numerous van der Waals contacts. ^{1}H NMR spectroscopic titrations in competitive and noncompetitive media, as well as binding studies in two-phase systems, such as dissolution of solid carbohydrates in apolar media and phase transfer of sugars from aqueous into organic solvents, revealed effective recognition of neutral carbohydrates and β- vs α-anomer binding preferences in the recognition of glycosides as well as significantly increased binding affinity of the receptors toward β-galactoside in comparison with the previously described receptors.

Introduction

Carbohydrate−protein interactions play a key role in a wide range of biological processes.[1,2a] The structural basis for selective carbohydrate recognition by carbohydrate-binding proteins has been intensively investigated by X-ray crystallography.[2] It has been shown that selectivity is achieved through a combination of hydrogen bonding to the sugar hydroxyl groups with hydrophobic packing, often including CH−π interactions between the sugar CH groups and aromatic amino acid side chains, such as indole of tryptophan and phenyl group of phenylalanine. Furthermore, numerous van der Waals contacts, involving all the atoms of the bound saccharides, were shown to contribute significantly to the binding affinity and selectivity.[2c] Examples of hydrogen-bonding interactions and van der Waals contacts in a protein−carbohydrate complex are shown in Figure 1. The participation

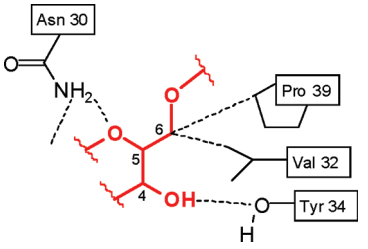

FIGURE 1. Examples of hydrogen bonds and van der Waals contacts in the complex of *Galanthus Nivalis Agglutinin* with Manα3(Manα6)Man.[2a,f]

(1) (a) Osborn, H.; Khan, T. *Oligosaccharides: Their Synthesis and Biological Roles*; Oxford University Press: New York, 2000. (b) Lindhorst, T. K. *Essentials of Carbohydrate Chemistry and Biochemistry*; Wiley-VCH: Weinheim, Germany, 2007. (c) Dwek, R. A.; Butters, T. D. *Chem. Rev.* **2002**, *102*, 283–284. (d) Gabius, H. J.; Siebert, H. C.; Andre, S.; Jimenez-Barbero, J.; Rudiger, H. *ChemBioChem* **2004**, *5*, 741–764. (e) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357–2364.

(2) (a) Lis, H; Sharon, N. *Lectins*; Kluwer Academic: Dordrecht, The Netherlands, 2003. (b) Lis, H; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674. (c) Quiocho, F. A. *Pure Appl. Chem.* **1989**, *61*, 1293–1306. (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) Wright, C. S.; Hester, G. *Structure* **1996**, *4*, 1339–1352.

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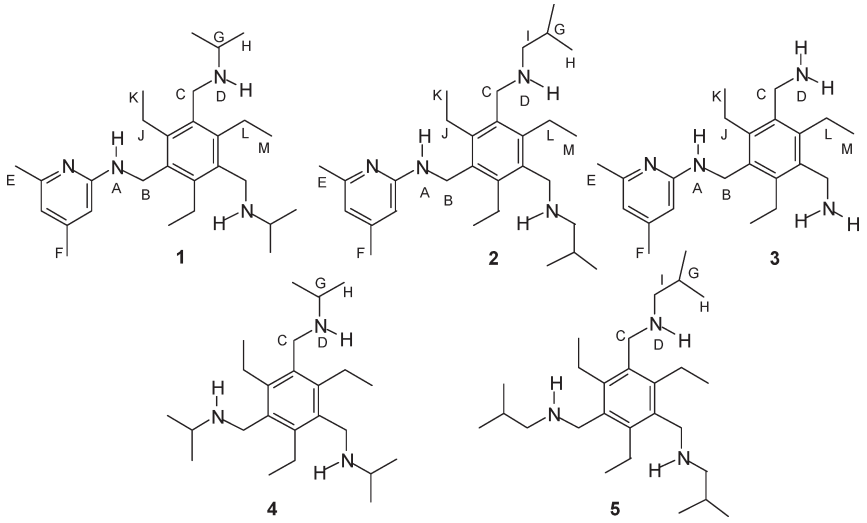

FIGURE 2. Structures of receptors **1**−**5**.

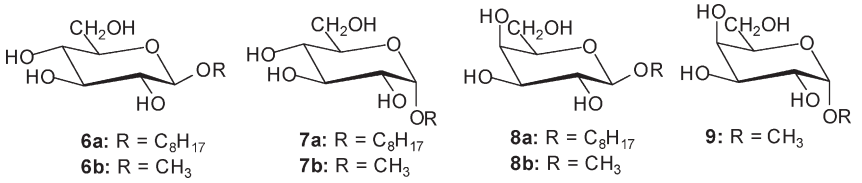

FIGURE 3. Structures of sugars investigated in this study.

of the primary amide group of asparagine and the isopropyl group of valine (see Figure 1) in the formation of hydrogen bonds and van der Waals contacts, respectively, has inspired the design of artificial receptor[3−5] **1** (see Figure 2), which was expected to be able to recognize a sugar molecule through a combination of hydrogen bonding, CH−π interactions,[6] and van der Waals contacts. Instead of the primary amide group shown in Figure 1, we have used the 2-aminopyridine unit, which can be regarded as a heterocyclic analogue of the asparagine/glutamine primary amide side chain and was shown to be an effective recognition group for carbohydrates.[7] The binding properties of **1** toward selected monosaccharides (see Figure 3) were compared with those of compounds **2**−**5** shown in Figure 2.

^{1}H NMR spectroscopic titrations in competitive and noncompetitive media, as well as binding studies in two-phase

(3) For reviews on carbohydrate recognition with artificial receptors using noncovalent interactions, see: (a) Davis, A. P.; James, T. D. In *Functional Synthetic Receptors*; Schrader, T., Hamilton, A. D., Eds., Wiley-VCH: Weinheim, Germany, 2005. (b) Davis, A. P.; Wareham, R. S. *Angew. Chem.* **1999**, *111*, 3161−3179; *Angew. Chem., Int. Ed.*, **1999**, *38*, 2979−2996. (c) Walker, D. B.; Joshi, G.; Davis, A. P. *Cell. Mol. Life Sci.* **2009**, *66*, 3177–3191. (d) Mazik, M. *Chem. Soc. Rev.* **2009**, *38*, 935–956. (e) Jin, S.; Cheng, Y.; Reid, S.; Li, M.; Wang, B. *Med. Res. Rev.* **2010**, *30*, 171–257.

(4) For examples of carbohydrate receptors operating through noncovalent interactions see refs 3 and 7 and: (a) Ferrand, Y.; Klein, E.; Barwell, N. P.; Crump, N. P.; Jiménez-Barbero, J.; Vicent, C.; Boons, G.-J.; Ingale, S.; Davis, A. P. *Angew. Chem., Int. Ed.* **2009**, *48*, 1775–1779. (b) Ardá, A.; Venturi, C.; Nativi, C.; Francesconi, O.; Gabrielli, G.; Cañada, F. J.; Jiménez-Barbero, J.; Roelens, S. *Chem. Eur. J.* **2010**, *16*, 414–418. (c) Abe, H; Takashima, S.; Yamamoto, T.; Inouye, M. *Chem. Commun.* **2009**, 2121–2123. (d) Abe, H.; Horii, A.; Matsumoto, S.; Shiro, M.; Inouye, M. *Org. Lett.* **2008**, *10*, 2685–2688. (e) Palde, P. B.; Gareiss, P. C.; Miller, B. L. *J. Am. Chem. Soc.* **2008**, *130*, 9566–9573. (f) Klein, E.; Ferrand, Y.; Auty, E. K.; Davis, A. P. *Chem. Commun.* **2007**, 2390–2392. (g) Nativi, C.; Cacciarini, M.; Francesconi, O.; Moneti, G.; Roelens, S. *Org. Lett.* **2007**, *9*, 4685–4688. (h) Ferrand, Y.; Crump, M. P.; Davis, A. P. *Science* **2007**, *318*, 619–622. (i) Klein, E.; Crump, M. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2005**, *44*, 298–302. (j) Abe, H.; Aoyagi, Y.; Inouye, M. *Org. Lett.* **2005**, *7*, 59–61. (k) Welti, R.; Abel, Y.; Gramlich, V.; Diederich, F. *Helv. Chim. Acta* **2003**, *86*, 548–562. (l) Welti, R.; Diederich, F. *Helv. Chim. Acta* **2003**, *86*, 494–503. (m) Wada, K.; Mizutani, T.; Kitagawa, S. *J. Org. Chem.* **2003**, *68*, 5123–5131. (n) Segura, M.; Bricoli, B.; Casnati, A.; Muñoz, E. M.; Sansone, F.; Ungaro, R.; Vicent, C. *J. Org. Chem.* **2003**, *68*, 6296–6303. (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. (p) Tamaru, S.-i.; Shinkai, S.; Khasanov, A. B.; Bell, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4972–4976. (q) Ladomenou, K.; Bonar-Law, R. P. *Chem. Commun.* **2002**, 2108–2109. (r) Bitta, J.; Kubik, S. *Org. Lett.* **2001**, *3*, 2637–2640. (s) Král, V.; Rusin, O.; Schmidtchen, F. P. *Org. Lett.* **2001**, *3*, 873–876. (t) Eblinger, F.; Schneider, H.-J. *Collect. Czech. Chem. Commun.* **2000**, *65*, 667–672.

(5) For reviews on boronic acid-based receptors, which use covalent interactions for sugar binding, see refs 3a,3e and: (a) James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159–200. (b) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem.* **1996**, *108*, 2038−2050; *Angew. Chem., Int. Ed.* **1996**, *35*, 1910−1922.For a recent example of a boronic acid-based receptor, see: (c) Phillips, M. D.; Fyles, T. M.; Barwell, N. P.; James, T. D. *Chem. Commun.* **2009**, 6557–6559. For a recent discussion on o-hydroxymethyl phenylboronic acid (benzoboroxole) binding capability, see: (d) Bérubé, M.; Dowlut, M.; Hall, D. G. *J. Org. Chem.* **2008**, *73*, 6471–6479.

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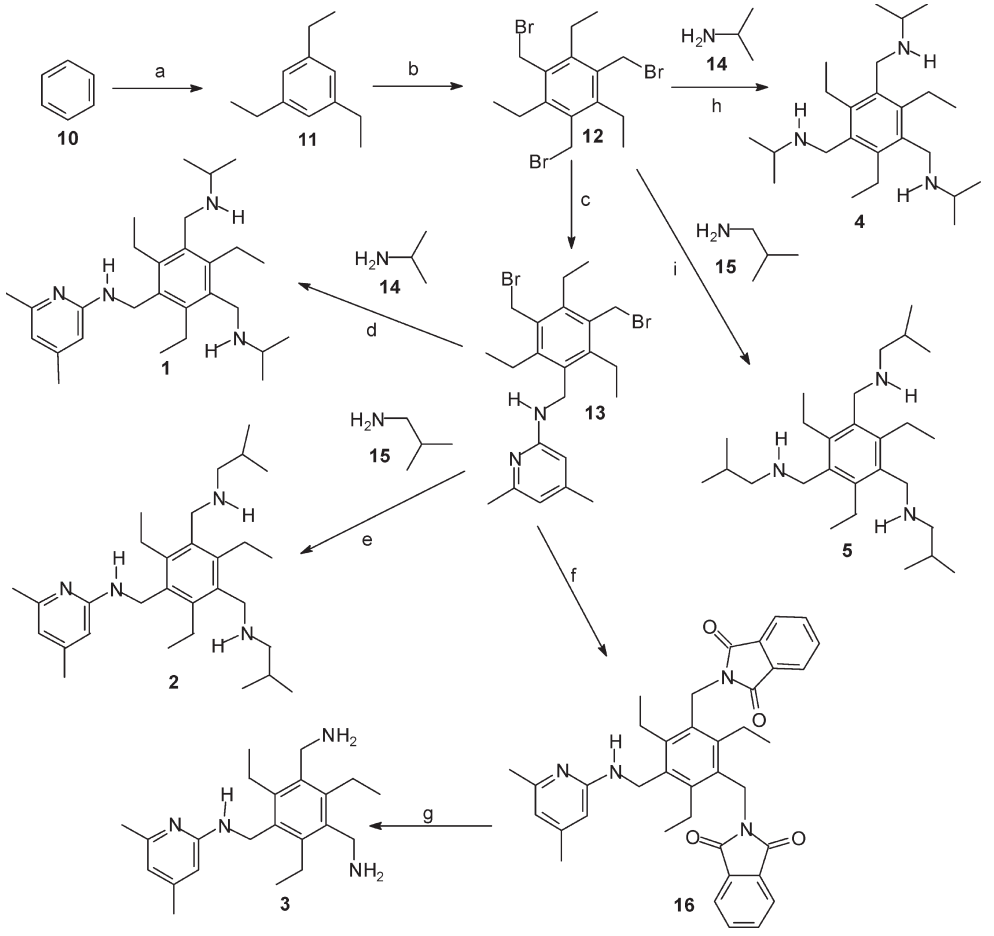
(7) (a) Mazik, M.; Hartmann, A. *Beilstein J. Org. Chem.*, **2010**, *6*, No. 9. (b) Mazik, M.; Hartmann, A.; Jones, P. G. *Chem. Eur. J.* **2009**, *15*, 9147–9159. (c) Mazik, M.; Buthe, A. C. *Org. Biomol. Chem.* **2009**, *7*, 2063–2071. (d) Mazik, M.; Hartmann, A. *J. Org. Chem.* **2008**, *73*, 7444–7450. (e) Mazik, M.; Buthe, A. C. *Org. Biomol. Chem.* **2008**, *6*, 1558–1568. (f) Mazik, M.; Kuschel, M. *Chem. Eur. J.* **2008**, *14*, 2405–2419. (g) Mazik, M.; Kuschel, M. *Eur. J. Org. Chem.* **2008**, 1517–1526. (h) Mazik, M.; Buthe, A. C. *J. Org. Chem.* **2007**, *72*, 8319–8326. (i) Mazik, M.; Cavga, H. *J. Org. Chem.* **2007**, *72*, 831–838. (j) Mazik, M.; König, A. *Eur. J. Org. Chem.* **2007**, 3271–3276. (k) Mazik, M.; Cavga, H. *Eur. J. Org. Chem.* **2007**, 3633–3638. (l) Mazik, M.; König, A. *J. Org. Chem.* **2006**, *71*, 7854–7857. (m) Mazik, M.; Cavga, H. *J. Org. Chem.* **2006**, *71*, 2957–2963. (n) Mazik, M.; Kuschel, M.; Sicking, W. *Org. Lett.* **2006**, *8*, 855–858. (o) Mazik, M.; Cavga, H.; Jones, P. G. *J. Am. Chem. Soc.* **2005**, *127*, 9045–9052. (p) Mazik, M.; Radunz, W.; Boese, R. *J. Org. Chem.* **2004**, *69*, 7448–7462. (q) Mazik, M.; Sicking, W. *Tetrahedron Lett.* **2004**, *45*, 3117–3121. (r) Mazik, M.; Radunz, W.; Sicking, W. *Org. Lett.* **2002**, *4*, 4579–4582. (s) Mazik, M.; Sicking, W. *Chem. Eur. J.* **2001**, *7*, 664–670. (t) Mazik, M.; Bandmann, H.; Sicking, W. *Angew. Chem.* **2000**, *112*, 562−565; *Angew. Chem., Int. Ed.* **2000**, *39*, 551−554.

SCHEME 1. Synthesis of Compounds 1−5[a]



[a]Key: (a) $AlCl_3$, CH_3CH_2Br, 0 °C to room temperature, 12 h (85%);[9] (b) 33% HBr in CH_3COOH, $ZnBr_2$, $(CH_2O)_n$, 90 °C, 16 h (94%); (c) 2 equiv of 2-amino-4,6-dimethylpyridine, CH_3CN/THF, K_2CO_3, room temperature, 3 days (20%); (d) 4 equiv of isopropylamine (**14**), THF/CH_3CN, K_2CO_3, room temperature, 2 days (75%); (e) 4 equiv of isobutylamine (**15**), THF/CH_3CN, K_2CO_3, room temperature, 2 days (71%); (f) potassium phthalimide, dimethyl sulfoxide, 95 °C, 8 h, (57%); (g) hydrazine hydrate, ethanol/toluene, reflux, 19 h, KOH (43%);[7b] (h) 3.3 equiv of isopropylamine (**14**), THF/CH_3CN, K_2CO_3, room temperature, 3 days (97%); (i) 3.3 equiv of isobutylamine (**15**) THF/CH_3CN, K_2CO_3, room temperature, 3 days (96%).

systems, such as dissolution of solid carbohydrates in apolar media and phase transfer of sugars from aqueous into organic solvents, revealed both effective recognition of neutral carbohydrates and interesting binding preferences[8] of the acyclic receptors **1** and **2**.

Results and Discussion

Synthesis of the Receptors. The basis for the synthesis of compounds **1**−**3** was 1,3-bis(bromomethyl)-5-[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (**13**).[7b] The reaction of **13** with isopropylamine (**14**) or isobutylamine (**15**) provided compounds **1** and **2**, respectively. The treatment of **13** with potassium phthalimide gave compound **16**, which was converted into 1,3-bis(aminomethyl)-5-[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (**3**) via a reaction with hydrazine. Compounds **4** and **5** were prepared through a reaction of 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene with isopropylamine (**14**) or isobutylamine (**15**), respectively. The synthesis of compounds **1**−**5** is summarized in Scheme 1.

Binding Studies in Two-Phase Systems: Liquid−Solid and Liquid−Liquid Extractions. Extractions of methyl pyranosides, such as β-glucoside **6b**, α-glucoside **7b**, β-galactoside **8b**, and α-galactoside **9**, from the solid state into a $CDCl_3$ solution[10] of receptor **1** or **2** (1 mM) provided evidence for stronger complexation of the β-anomers **6b** and **8b** (see Table 1). The preference of **1** and **2** for β- vs α-glycoside indicated by liquid−solid extractions was further confirmed by ^1H NMR spectroscopic titrations (see below). The extraction experiments indicated that the isopropylamino-based compound **1** is a more powerful monosaccharide receptor than **2**, containing isobutylamino groups. In comparison to the receptors **1** and **2**, the extraction experiments indicated a lower level of affinity of compounds **3**−**5** toward the tested monosaccharides (see Table 1).

(8) For a discussion on selectivity in supramolecular host−guest complexes, see: Schneider, H.-J.; Yatsimirsky, A. *Chem. Soc. Rev.* **2008**, *37*, 263–277.

(9) Wallace, K. J.; Hanes, R.; Anslyn, E.; Morey, J.; Kilway, K. V.; Siegel, J. *Synthesis* **2005**, 2080–2083.

(10) The dissolution of solid carbohydrates in apolar media provides valuable means of studying carbohydrate recognition by organic-soluble receptors. For examples of receptors which are able to dissolve solid carbohydrates in apolar media, see refs 3a, 4b, and 7a,7b,7p,7r and: (a) Bähr, A.; Felber, B.; Schneider., K.; Diederich, F. *Helv. Chim. Acta* **2000**, *83*, 1346–1376. (b) Inouye, M.; Chiba, J.; Nakazumi, H. *J. Org. Chem.* **1999**, *64*, 8170–8176. (c) Inouye, M.; Miyake, T.; Furusyo, M.; Nakazumi, H. *J. Am. Chem. Soc.* **1995**, *117*, 12416.

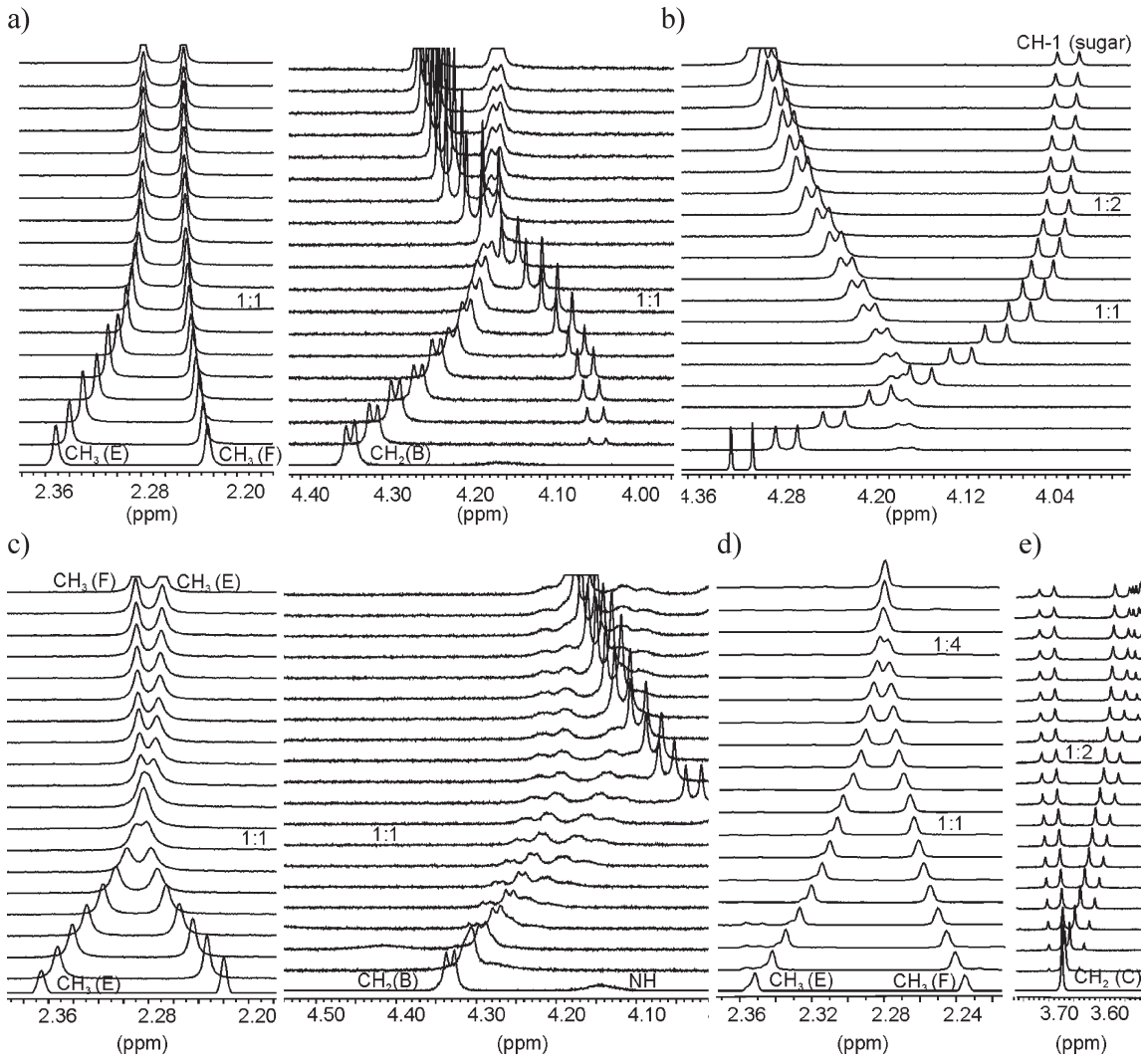


FIGURE 4. (a, c) Partial ^{1}H NMR spectra (400 MHz; $CDCl_3$) of receptor **1** before (bottom) and after the addition of β-glucoside **6a** (a) and β-galactoside **8a** (c): [**1**] = 0.97 mM, 0.00−4.80 equiv of **6a** or **8a**. (b) Partial ^{1}H NMR spectra of sugar **6a** before (bottom) and after the addition of receptor **1** (inverse titration): [**6a**] = 0.78 mM, 0.00−4.99 equiv of **1**. (d, e) Partial ^{1}H NMR spectra of receptors **3** (d) and **4** (e) before (bottom) and after the addition of β-galactoside **8a** (b): [**3**] = 0.96 mM, 0.00−4.85 equiv of **8a**; [**4**] = 0.91 mM, 0.00−4.48 equiv of **8a**.

TABLE 1. Solubilization of Sugars in $CDCl_3$ by Receptors 1−5 (1 mM Solutions)

sugar	sugar/**1**[a]	sugar/**2**[a]	sugar/**3**[a]	sugar/**4**[a]	sugar/**5**[a]
β-D-glucoside **6b**	0.79	0.60	0.34	0.41	0.24
α-D-glucoside **7b**	0.41	0.21	0.14	0.15	0.10
β-D-galactoside **8b**	0.84	0.66	0.30	0.43	0.24
α-D-galactoside **9**	0.38	0.30	0.23	0.24	0.20

[a]Molar ratios of sugar to receptor occurring in solution (the ^{1}H NMR signals of the corresponding sugar were integrated with respect to the receptor's signals to provide the sugar to receptor ratio; control experiments were performed in the absence of the receptor).

We were interested to see whether **1** would be able to extract carbohydrates from aqueous solution into nonpolar solvent.[11] Studies of the extraction (using the procedure described by Davis et al.; see refs 11a, 11b and see also ref 7b) of methyl β-D-glucoside (**6b**) and methyl β-D-galactoside (**8b**) from aqueous solution into chloroform revealed that compound **1** (1 mM chloroform solution) is capable of extracting about 0.15 equiv of β-glucoside **6b** and β-galactoside **8b** from 1 M aqueous solutions (control experiments were performed in the absence of the receptor).

Binding Studies in Homogeneous Solution. The interactions of the receptors and carbohydrates were investigated by ^{1}H NMR spectroscopic titrations in $CDCl_3$ and DMSO-d_6/$CDCl_3$ mixtures. The stoichiometry of the receptor−sugar complexes was determined by mole ratio plots[12] (for examples, see the Supporting Information) and by a curve-fitting analysis of the titration data.[13−15]

(11) For examples of macrocyclic receptors which are able to extract sugars from water into nonpolar organic solutions, see: (a) Velasco, T.; Lecollinet, G.; Ryan, T.; Davis, A. P. *Org. Biomol. Chem.* **2004**, *2*, 645–647. (b) Ryan, T.; Lecollinet, G.; Velasco, T.; Davis, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4863–4866. (c) Aoyama, Y.; Tanaka, Y.; Sugahara, S. *J. Am. Chem. Soc.* **1989**, *111*, 5397. (d) Aoyama, Y.; Tanaka, Y.; Toi, H.; Ogoshi, H. *J. Am. Chem. Soc.* **1988**, *110*, 634.

(12) For a description of the mole ratio method, see: (a) Schneider, H.-J.; Yatsimirsky, A. *Principles and Methods in Supramolecular Chemistry*; Wiley: Chichester, U.K., 2000; p 148. (b) 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.

TABLE 2. Association Constants[a,b] for Receptors 1−5 and Carbohydrates 6a−8a

host−guest complex	solvent	K_{11} (M^{-1})	K_{21} or K_{12} (M^{-1})	$\beta_{21} = K_{11}K_{21}$ or $\beta_{12} = K_{11}K_{12}$ (M^{-2})
1·6a	$CDCl_3$	28800	530[c]	1.52×10^7
	5% DMSO-d_6/$CDCl_3$	2550	190[d]	4.85×10^5
1·7a	$CDCl_3$	4360	210[d]	9.15×10^5
1·8a	$CDCl_3$	44540	1680[c]	7.48×10^7
	5% DMSO-d_6/$CDCl_3$	3830	300[d]	1.15×10^6
2·6a	$CDCl_3$	12600	450[c]	5.67×10^6
2·7a	$CDCl_3$	1660	280[d]	4.64×10^5
2·8a	$CDCl_3$	19400	940[c]	1.82×10^7
3·6a	$CDCl_3$	4580	150[c]	6.87×10^5
3·8a	$CDCl_3$	5950	310[c]	1.84×10^6
4·6a	$CDCl_3$	4420	220[c]	9.72×10^5
4·8a	$CDCl_3$	5200	340[c]	1.76×10^6
5·6a	$CDCl_3$	2800	260[c]	7.28×10^5

[a]Average K_a values from multiple titrations in $CDCl_3$. [b]Errors in K_a are less than 20%. [c]K_{21} corresponds to 2:1 receptor−sugar association constant. [d]K_{12} corresponds to 1:2 receptor−sugar association constant.

Addition of octyl pyranosides **6a**−**8a** to a $CDCl_3$ solution of **1**−**5** (the concentration of receptor was kept constant and that of the corresponding sugar was varied) caused various changes in the ^{1}H NMR spectrum of the receptor. In particular, the signal due to the NH^A of **1**−**3** moved significantly downfield (in the range of 1.2−2.6 ppm, see Table S4 in the Supporting Information), while those due to CH_2^B and CH_3^E moved upfield (further changes in chemical shift are given in Table S4; see also Figure 4 and Figure S1 in the Supporting Information). In some cases splitting of the signals due to the CH_2^B, CH_2^C, or CH_3^H group was observed, as given in Table S4. The complexation-induced chemical shifts of the NH^A and $CH_3^{E,F}$ protons (for labeling, see Figure 2) were monitored for the determination of the binding constants, which are summarized in Table 2. In addition to the 1:1 complexes, binding constants for complexes of higher stoichiometry (1:2 or 2:1 receptor−sugar complexes) were measured; the values of K_{12} or K_{21} are considerably smaller than those of K_{11} (see Table 2). The binding studies showed that the interactions of **1** and **2** with β-glycosides **6a** and **8a** are more favorable than those with α-glucoside **7a**. The interactions of **6a**−**8a** with receptor **1** were shown to be stronger than those with **2**. In addition, the binding affinities of **1** and **2** toward **6a**/**8a** were shown to be significantly higher than those of **3** (see Table 2 and Figure 4d), indicating an important role of the isopropyl or isobutyl groups in the complex formation. When the aminopyridine group in **1**/**2** was replaced by the isopropylamino (compound **4**) or isobutylamino group (compound **5**), the expected decrease in the binding affinity to the tested glycosides was observed (see Table 2). Compound **4**, which was found to be a more effective receptor than the isobutylamino-based compound **5**, showed affinities toward β-glucoside **6a** and β-galactoside **8a** similar to those of compound **3**. It should be also noted that the symmetrical isopropylamino-based compound **4**, possessing only three NH groups as hydrogen bonding sites, showed a significantly decreased affinity (about 10 times lower) to β-glucoside **6a** but a level of affinity toward β-galactoside **8a** similar to that of the previously described[7p] symmetrical aminopyridine-based receptor (K_{11} = 5200 M^{-1} for **4·8a** vs K_{11} = 3070 M^{-1} for the previously described receptor[7p] and **8a**).

Since the binding constants for **1·6a** and **1·8a** in $CDCl_3$ were determined to be higher than 1×10^4 M^{-1}, additional ^{1}H NMR titrations in a more polar solvent (DMSO-d_6/$CDCl_3$ mixture) were carried out (for a review discussing the limitations of the NMR spectroscopy method, see ref 16). The best fit of the titration data was obtained with the "mixed" 1:1 and 1:2 receptor−sugar binding model; the results are given in Table 2. As expected for the recognition of polar molecules, the affinities decreased as the solvent polarity increased.[17]

The interactions between the monosaccharides **6a**/**8a** and the receptors **1**/**2** were also investigated on the basis of inverse titrations in which the concentration of the sugar was held constant and that of the receptor varied. During the titration of **6a**/**8a** with **1** or **2** in $CDCl_3$, the signals due to the OH protons of **6a** and **8a** shifted downfield with strong broadening and became almost indistinguishable from the baseline after the addition of only 0.1 equiv of the receptor, indicating the participation of the sugar OH groups in the formation of hydrogen bonds. The addition of **1** or **2** to a $CDCl_3$ solution of **6a** or **8a**, furthermore, caused significant upfield shifts of the sugar CH signals (see, for example Figure 4b), indicating the participation of the sugar CH units in the formation of the CH···π interactions with the

(13) (a) Wilcox, C. S.; Glagovich, N. M. *Program HOSTEST 5.6*; University of Pittsburgh, Pittsburgh, PA, **1994**. (b) Cowart, M. D.; Sucholeiki, I.; Bukownik, R. R.; Wilcox, C. S. *J. Am. Chem. Soc.* **1988**, *110*, 6204–6210. (c) The HOSTEST 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 (1:1, 1:2, or 2:1 receptor−substrate complex), and "mixed" binding models containing more than one type of complex in solution (for example, 1:1 and 1:2 or 1:1 and 2:1 receptor−substrate complex).

(14) Hynes, M. J. *J. Chem. Soc., Dalton Trans.* **1993**, 311–312.

(15) (a) The titration data were analyzed by non-linear regression analysis, using the program HOSTEST 5.6[13] and EQNMR.[14] (b) The error in K_a was < 20%. (c) K_{11} corresponds to the 1:1 association constant. K_{21} corresponds to the 2:1 receptor−sugar association constant. K_{12} corresponds to the 1:2 receptor−sugar association constant. $\beta_{21} = K_{11} \times K_{21}$, $\beta_{12} = K_{11} \times K_{12}$.

(16) Fielding, L. *Tetrahedron* **2000**, *56*, 6151–6170.

(17) For a discussion on solvent effects in carbohydrate binding by synthetic receptors, see: Klein, E.; Ferrand, Y.; Barwell, N. P.; Davis, A. P. *Angew. Chem.* **2008**, *120*, 2733−2736; *Angew. Chem., Int. Ed.*, **2008**, *47*, 2693−2696.

(18) For discussions on the importance of carbohydrate−aromatic interactions, see: (a) Tsuzuki, S.; Uchimaru, T.; Mikami, M. *J. Phys. Chem. B* **2009**, *113*, 5617–5621. (b) Terraneo, G.; Potenza, D.; Canales, A.; Jiménez-Barbero, J.; Baldridge, K. K.; Bernardi, A. *J. Am. Chem. Soc.* **2007**, *129*, 2890–2900. (c) Chávez, M. I.; Andreu, C.; Vidal, P.; Aboitiz, N.; Freire, F.; Groves, P.; Asensio, J. L.; Asensio, G.; Muraki, M.; Cañada, F. J.; Jiménez-Barbero, J. *Chem. Eur. J.* **2005**, *11*, 7060–7074. (d) Screen, J.; Stanca-Kaposta, E. C.; Gamblin, D. P.; Liu, B.; Macleod, N. A.; Snoek, L. C.; Davis, B. G.; Simons, J. P. *Angew. Chem., Int. Ed.* **2007**, *46*, 3644–3648. (e) Kiehna, S. H.; Laughrey, Z. R.; Waters, M. L. *Chem. Commun.* **2007**, 4026–4028. (f) Morales, J. C.; Penadés, S. *Angew. Chem., Int. Ed.* **1998**, *37*, 654–657.

benzene ring of the corresponding receptor (for discussions on the importance of carbohydrate−aromatic interactions, see ref 18; for examples of CH−π interactions in the crystal structures of the complexes formed between artificial receptors and carbohydrates, see ref 7o). Among the CH signals, the signal due to the 2-CH proton of **6a** and **8a** showed the largest shift (for example, 1.7 and 2.1 ppm for the titration **6a**·**1** and **8a**·**1**, respectively). Analysis of the data supported the "mixed" 1:1 and 1:2 sugar−receptor binding model and provided binding constants which are identical, within the limits of uncertainty, with those determined from titrations where the roles of receptor and substrate were reversed.

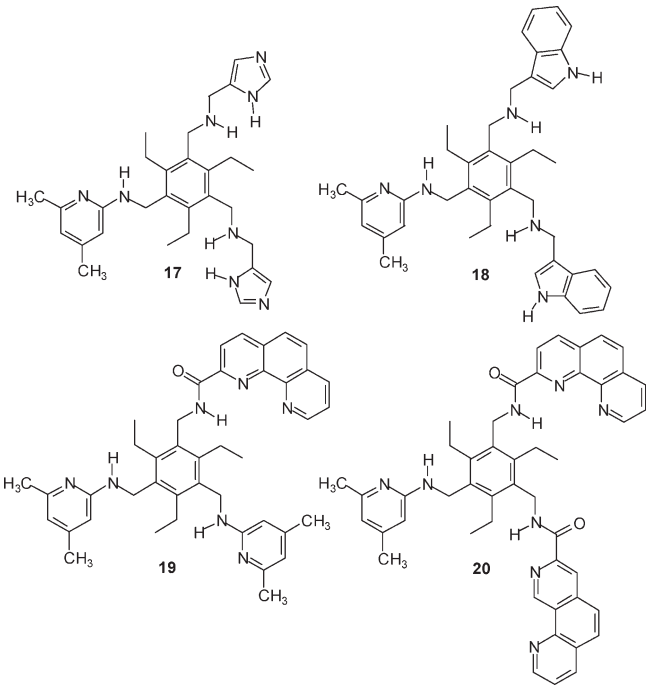


FIGURE 5. Structures of the recently described receptors **17**/**18**[7a] showing β-galactoside vs β-glucoside as well as β- vs α-anomer binding preferences and receptors **19**/**20**[7b,d] showing α- vs β-anomer binding preferences in the recognition of glycosides.

In comparison to the previously described symmetrical, three-armed aminopyridine-based receptor,[7p] which shows a β-glucoside vs β-galactoside binding preference, compounds **1** and **2** exhibit an inverse preference and a significantly higher binding affinity toward the β-galactoside **8** (about 10 times higher in the case of **1**). It should be also noted that an enhancement of the binding affinity toward β-galactoside was recently observed for the imidazole/aminopyridine- and indole/aminopyridine-based receptors **17** and **18** (see Figure 5).[7a] In contrast to these results, the phenanthroline/aminopyridine-based receptors **19** and **20** show a high binding affinity toward α-galactoside **9** ($K_{11} > 10^5$ M^{-1} in $CDCl_3$) as well as high α- vs β-galactoside preference.[7b,d]

Molecular Modeling. According to molecular modeling calculations the 1:1 complex between receptor **1** and β-galactoside **8b** can be stabilized by at least six hydrogen bonds (NH···O and OH···N hydrogen bonds, see Table 3) and interactions of sugar 2-CH with the central phenyl group of the receptor molecule. Furthermore, CH···O and CH···N interactions (as indicated by the calculations, some of these interactions can be of hydrogen bonding type) provide an additional stabilization of the receptor-sugar complex (see

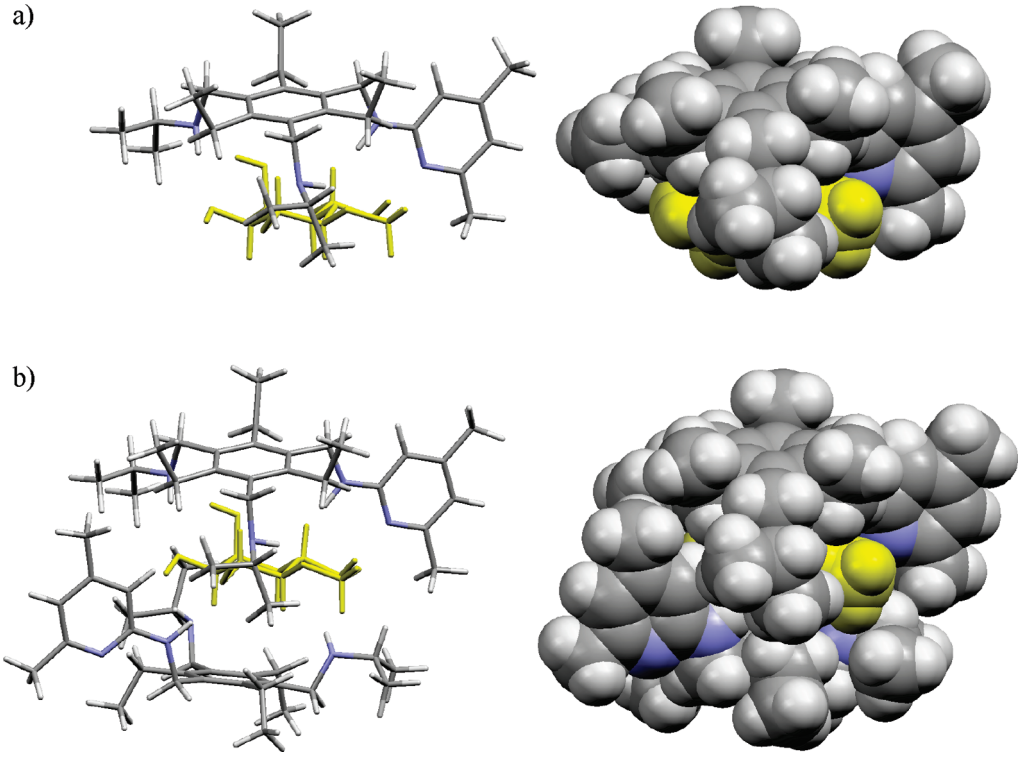


FIGURE 6. Energy-minimized structure of the 1:1 (a) and 2:1 complexes (b) formed between receptor **1** and methyl β-galactoside **8b** (MacroModel V.8.5, OPLS-AA force field, MCMM, 50 000 steps): (gray) receptor C; (blue) receptor N; (yellow) sugar molecule.

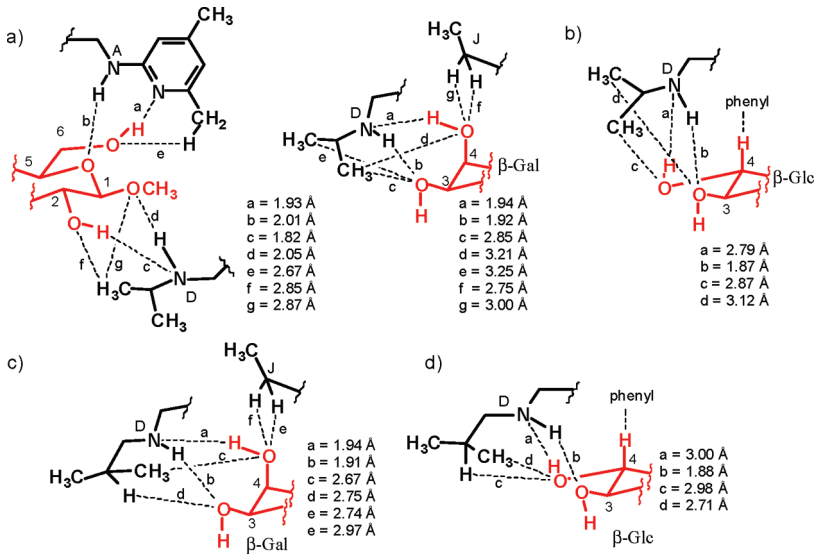


FIGURE 7. Examples of noncovalent interactions (hydrogen bonding and van der Waals contacts) indicated by molecular modeling studies in 1:1 complexes of receptor **1** with β-galactoside **8b** (a) and β-glucoside **6b** (b) as well as in 1:1 complexes of receptor **2** with β-galactoside **8b** (c) and β-glucoside **6b** (d) (MacroModel V.8.5, OPLS-AA force field, MCMM, 50 000 steps).

TABLE 3. Examples of Noncovalent Interactions Indicated by Molecular Modeling Calculations[a] for the Complexes Formed between Receptor 1 and Sugar 8b

1:1 receptor−sugar complex	2:1 receptor−sugar complex[b]
$HN^D\cdots$HO-2; $NH^D\cdots O-CH_3$	(I) $HN^D\cdots$HO-2; (I) $NH^D\cdots O-CH_3$
$CH(CH_3)_2{}^H\cdots OCH_3$	(I) $CH(CH_3)_2{}^H\cdots$OH-2
$CH(CH_3)_2{}^H\cdots$OH-2	(I) $NH^D\cdots$OH-3; (I) $HN^D\cdots$HO-4
$HN^D\cdots$HO-4	(I) pyridine-N$\cdots$HO-6
$NH^D\cdots$OH-3	(I) $NH^A\cdots$O-ring
$CH(CH_3)_2{}^H\cdots$OH-3	(I) pyridine-$CH_3{}^E\cdots$OH-6
$CH_3CH_2{}^J\cdots$OH-4	(I) phenyl$\cdots$HC-6; (I) phenyl$\cdots$HC-2
$CH_3CH_2{}^L\cdots$OH-3	(II) $NH^D\cdots$OH-6
pyridine-N$\cdots$HO-6	(II) $NH^A\cdots$OH-2; (II) $HN^A\cdots$HO-3
$NH^A\cdots$O-ring	(II) $(CH_3)_2CH^G\cdots$OH-6
pyridine-N$\cdots H_3CO$	(II) phenyl$\cdots$HC-1; (II)phenyl$\cdots$HC-3
pyridine-$CH_3{}^E\cdots$OH-6	(II) phenyl$\cdots$HC-5
phenyl$\cdots$HC-2	(II) $HN^D\cdots$HC-4

[a]MacroModel V.8.5, OPLS-AA force field, MCMM, 50 000 steps. [b]I and II denote two receptors in the 2:1 receptor−sugar complex; for labeling see Figure 2.

Table 3 and Figure 6a). In the case of **1**·**8b**, the 4-OH group of the β-galactoside **8b** seems to be better positioned in the binding pocket of **1** than the 4-OH of the β-glucoside **6b** in the **1**·**6b** complex (for comparison, see Figure 7a,b).

In the case of a 2:1 receptor−sugar complex between **1** and **8b**, the two receptor molecules almost completely enclose the sugar, leading to involvement of all sugar hydroxyl groups in interactions with the two receptor molecules (see Table 3 and Figure 6b). The OH groups are involved in the formation of cooperative hydrogen bonds, which result from the simultaneous participation of a sugar OH as donor and acceptor of hydrogen bonds; the phenyl groups of both receptors stack on the sugar ring, and both sides of the pyranose ring are involved in CH$\cdots\pi$ interactions (see Table 3 and Figure 6b).

The preference of **2** for β-galactoside vs β-glucoside shown by ^{1}H NMR titrations was also indicated by molecular modeling calculations (for examples of noncovalent interactions in **2**·**8b** and **2**·**6b**, see Figure 7c,d).

Conclusions

Crystal structures of protein−-carbohydrate complexes revealed the participation of the isopropyl/isobutyl side chain of valine/leucine in van der Waals contacts with the carbohydrate substrates. We were interested to see whether isopropyl and isobutyl groups would be suitable building blocks for artificial carbohydrate receptors. Compounds **1** and **2**, containing a trisubstituted triethylbenzene core, were expected to recognize sugar molecules through a combination of NH$\cdots$O and OH$\cdots$N hydrogen bonds, CH$\cdots\pi$ interactions,[6] and numerous van der Waals contacts. ^{1}H NMR spectroscopic titrations and binding studies in two-phase systems, such as the dissolution of solid carbohydrates in apolar media, revealed effective recognition of neutral carbohydrates, β- vs α-anomer binding preferences in the recognition of glycosides, and considerably increased binding affinity toward β-galactoside in comparison with the previously described symmetrical aminopyridine-based receptor[7p] and other acyclic receptors.[7f] Although 1:1 complexes predominate in the solution, the presence of 1:2 or 2:1 receptor−sugar complexes, depending on the titration conditions, was also detected. Compound **1**, containing isopropylamino groups, was shown to be a more effective carbohydrate receptor than the isobutylamino-based compound **2**. Liquid−liquid extractions demonstrated ability of **1** to extract monosaccharides from water into chloroform; such an ability is interesting, considering that the receptor possesses a very simple, acyclic structure. In comparison to the previously described receptor, incorporating three aminopyridine-based recognition units,[7p] receptor **1** showed significantly increased affinity to β-galactoside (about 10 times higher affinity) but decreased affinity toward β-glucoside (about 2 times lower).

The affinities of **3** for the tested monosaccharides were shown to be considerably lower than those of **1** and **2**. Tighter binding of monosaccharides by **1**/**2** compared to **3** has been attributed to van der Waals contacts between the monosaccharide substrate and the isopropyl/isobutyl groups, which are absent in **3**. The replacement of the aminopyridine group

in **1** and **2** by an isopropylamino or isobutylamino unit, respectively, results in a decrease in the binding constants. The affinity of the symmetrical isopropylamino-based receptor **4** toward the selected β-glycosides was shown to be similar to that of **3** but higher than that of **5**. In comparison to the previously described symmetrical aminopyridine-based receptor,[7p] compound **4**, possessing only three NH groups as hydrogen bonding sites, showed a significantly decreased affinity (about 10 times lower) to β-glucoside **6a** but a similar affinity toward β-galactoside **8a**. Considering the simple structure of **4**,[19] the binding affinity toward β-galactoside is noteworthy.

Similar to our previous studies,[7a,b] the binding studies with compounds **1**−**3** demonstrated that, depending on the nature of the recognition units used as the building blocks for the acyclic receptor structures, effective carbohydrate receptors with different binding preferences can be generated. The exact prediction of the binding preference is still further away, and it is hoped that systematic studies toward recognition units for carbohydrates will contribute significantly to the solution of this unsolved problem. In this context, the acyclic receptors represent particularly interesting objects for such systematic studies. It should be noted that artificial carbohydrate receptors using noncovalent interactions for sugar binding provide valuable model systems to study the underlying principles of carbohydrate-based molecular recognition processes and may serve as a basis for the development of saccharide sensors or new therapeutics.[20]

Experimental Section

Analytical TLC was carried out on silica gel 60 F_{254} plates employing chloroform/methanol mixtures as the mobile phase. Melting points are uncorrected. Sugars **6**−**9** are commercially available. The binding studies are described in the Supporting Information.

General Procedure for the Synthesis of Compounds 1 and 2. To a mixture of isopropylamine (**14**; 0.21 mL, 0.15 g, 2.49 mmol) or isobutylamine (**15**; 0.26 mL, 0.18 g, 2.49 mmol), CH_3CN (20 mL), and K_2CO_3 (0.18 g, 1.29 mmol) was added dropwise a THF/CH_3CN (25 mL, 4:1) solution of 1,3-bis(bromomethyl)-5-[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (**13**; 0.30 g, 0.62 mmol). After complete addition, the mixture was stirred at room temperature for 24 h. The solvents were then removed under vacuum; the crude product was purified by column chromatography (silica gel, $CHCl_3/CH_3OH$, 7:1).

1,3-Bis[(isopropylamino)methyl]-5-[(4,6-dimethylpyridin-2-yl)-aminomethyl]-2,4,6-triethylbenzene (1). Yield: 75%. Mp: 39−41 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 0.07 M): δ 1.13 (d, J = 6.3 Hz, 12 H), 1.19−1.27 (m, 9 H), 2.21 (s, 3 H), 2.35 (s, 3 H), 2.76 (q, J = 7.6 Hz, 4 H), 2.82 (q, J = 7.6 Hz, 2 H), 2.93 (sept, J = 6.3 Hz, 2 H), 3.71 (s, 4 H), 4.28 (br s, 1 H), 4.34 (d, J = 4.0 Hz, 2 H), 6.06 (s, 1 H), 6.32 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.8, 21.1, 22.7, 24.2, 40.6, 45.4, 50.1, 103.6, 113.6, 132.6, 134.3, 142.42, 142.6, 148.6, 156.6, 158.3 ppm. HR-MS (EI): m/z calcd for $C_{28}H_{46}N_4$ 438.372 25, found 438.372 27. R_f = 0.10 ($CHCl_3$/CH_3OH, 7:1 v/v).

1,3-Bis[(isobutylamino)methyl]-5-[(4,6-dimethylpyridin-2-yl)-aminomethyl]-2,4,6-triethylbenzene (2). Yield: 71%. Mp: 42−44 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 0.03 M): δ 0.93 (d, J = 6.6 Hz, 12 H), 1.20−1.28 (m, 9 H), 1.77 (sept, J = 6.6 Hz, 2 H), 2.22 (s, 3 H), 2.35 (s, 3 H), 2.54 (d, J = 6.7 Hz, 4 H), 2.76 (q, J = 7.6 Hz, 4 H), 2.83 (q, J = 7.6 Hz, 2 H), 3.68 (s, 4 H), 4.18 (br s, 1 H), 4.34 (d, J = 4.20 Hz, 2 H), 6.07 (s, 1 H), 6.33 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.8, 16.9, 20.8, 21.1, 21.4, 22.7, 24.2, 28.4, 40.7, 48.1, 59.2, 103.4, 113.6, 132.5, 134.8, 142.4, 142.7, 148.6, 156.7, 158.4 ppm. HR-MS (EI): m/z calcd for $C_{30}H_{50}N_4$ 466.403 55, found 466.403 57. R_f = 0.15 ($CHCl_3/CH_3OH$, 7:1 v/v).

General Procedure for the Synthesis of Compounds 4 and 5. To a mixture of isopropylamine (**14**; 0.32 mL, 3.74 mmol) or isobutylamine (**15**; 0.36 mL, 3.74 mmol), CH_3CN (30 mL), and K_2CO_3 (0.5 g) was added dropwise a THF/CH_3CN (40 mL, 4:1) solution of 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene (**12**; 0.5 g, 1.13 mmol). After complete addition, the mixture was stirred at room temperature for 36 h. The solvents were then removed under vacuum; the crude product was washed several times with water and dried.

1,3,5-Tris[(isopropylamino)methyl]-2,4,6-triethylbenzene (4). Yield: 97%. Mp: 69−70 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 (d, J = 6.3 Hz, 18 H), 1.23 (t, J = 7.5 Hz, 9 H), 2.79 (q, J = 7.5 Hz, 6 H), 2.91 (sept, J = 6.3 Hz, 3 H), 3.67 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.9, 22.6, 22.9, 45.7, 49.8, 134.5, 141.7. HR-MS (EI): m/z calcd for $C_{24}H_{45}N_3$ 375.361 34, found 375.361 28. R_f = 0.10 ($CHCl_3/CH_3OH$, 5:1 v/v).

1,3,5-Tris[(isobutylamino)methyl]-2,4,6-triethylbenzene (5). Yield: 96%. Mp: 38−39 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (d, J = 6.7 Hz, 18 H), 1.24 (t, J = 7.5 Hz, 9 H), 1.77 (sept, J = 6.7 Hz, 3 H), 2.54 (d, J = 6.7 Hz, 6H), 2.80 (q, J = 7.5 Hz, 6 H), 3.67 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.9, 20.8, 22.6, 28.3, 48.2, 59.2, 134.5, 141.9. HR-MS (ESI): m/z calcd for $C_{27}H_{52}N_3$ (M^+ + H) 418.416 12, found 418.416 03. R_f = 0.65 ($CHCl_3/CH_3OH$, 5:1 v/v).

Acknowledgment. Financial support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

Supporting Information Available: Copies of the ^{1}H and ^{13}C NMR spectra of **1**, **2**, **4**, and **5** (Figures S4−S12), representative mole ratio plots (Figures S2 and S3), and further examples of ^{1}H NMR titrations (Figures S1a and S1b), descriptions of the ^{1}H NMR titration experiments (Tables S1−S3), and changes in chemical shift observed during ^{1}H NMR titrations of compounds **1**−**3** with sugars **6a**−**8a** in $CDCl_3$ (Table S4). This material is available free of charge via the Internet at http://pubs.acs.org.

(19) Artificial carbohydrate receptors very closely related to ours have been recently presented, although binding constants in $CDCl_3$ were only in the range of 58−465 M^{-1}; see: Fernandez-Trillo, F.; Fernandez-Megia, E.; Riguera, R. *J. Org. Chem.* **2010**, *75*, 3878–3881.

(20) (a) Davis, A. P. *Org. Biomol. Chem.* **2009**, *7*, 3629–3638. (b) Kubik, S. *Angew. Chem.* **2009**, *121*, 1750−1753; *Angew. Chem., Int. Ed.*, **2009**, *48*, 1722−1725. (c) Mazik, M. *ChemBioChem* **2008**, *9*, 1015–1017.