Highly effective receptors showing di- *vs.* monosaccharide preference†

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Receptors **1** and **2**, incorporating two heterocyclic recognition units as well as oxime- or hydroxymethyl-based hydrogen-bonding sites, were prepared, and their binding properties toward neutral sugars were determined. The design of these receptors was inspired by the binding motifs observed in the crystal structure of protein–carbohydrate complexes. The receptors **1** and **2** are able to recognize both mono- and disaccharides, with a strong preference for the disaccharides. Both hydrogen-bonding and interactions of the sugar CH's with the phenyl rings of the receptor contribute to the stabilisation of the receptor–sugar complexes. Molecular modeling calculations, synthesis and binding studies are described.

Introduction

The interactions observed in the crystal structures of protein–carbohydrate complexes[1] (for examples, see Fig. 1) inspire the development of different artificial receptor structures for the recognition of carbohydrates.[2–5] Our previous studies showed that acyclic receptors containing two to four recognition units interconnected by a phenyl, biphenyl or diphenylmethane spacer perform effective recognition of carbohydrates through multiple interactions.[5] Depending on the nature and number of recognition units and connecting bridges used as the building blocks (see Fig. 2), a variety of structures with different binding properties could be obtained.

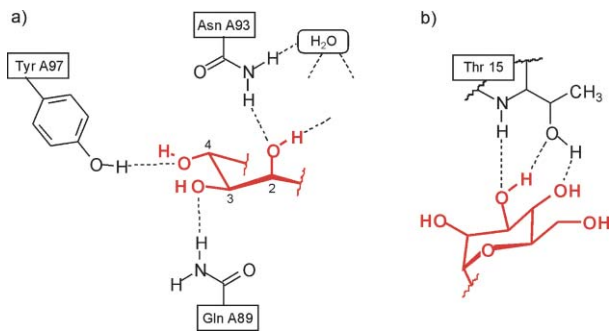

Fig. 1 Examples of hydrogen bonds in the complexes of (a) *Galanthus nivalis* lectin with mannose[1f,1a] and (b) concanavalin A with Manα6(Manα3)Man.[1e,1g]

In this study, we focused on interactions of receptors **1** and **2** (see Fig. 3 and 4) with neutral sugar molecules. The design of the receptor **1**, containing suitably positioned amine and hydroxymethyl units, as well as 2-aminopyridine groups[6] (as heterocyclic analogues of the asparagine/glutamine primary amide side chains[7]), was inspired by the binding motifs shown in Fig. 1. As in natural complexes, the participation of different types of hydrogen-bonding groups in the recognition process was expected to be favorable for reaching high binding affinity and selectivity of the receptor. It should also be noted that the natural recognition unit consisting of main chain amide of Ser or Thr and side chain hydroxyl of the same amino acid (see Fig. 1b) has been successfully mimicked with an aromatic analogue, which was used for the construction of receptors showing selectivity for *N*-acetylneuraminic acid over glucuronic acid in competitive media like DMSO or water–DMSO.[5e]

Recently, we have shown that three-armed oxime-based receptors are able to bind neutral sugar molecules in chloroform and water-containing chloroform solutions with high affinity.[5c] The using of the oxime groups as hydrogen bonding sites for carbohydrates was inspired by the interactions involving pairs of OH···N hydrogen bonds, which were observed between oxime functionalities in the crystal structures of different oxime molecules.[8] Molecular modeling calculations indicated that combining oxime- and aminopyridine-based recognition units, as in the case of the compound **2**, should cause further improvement of the binding affinity of the new receptor.

As in previously described artificial systems,[5] the participation of the phenyl rings of the receptors **1** and **2** in the interactions with sugar CH's was expected to provide additional stabilization of the receptor–sugar complexes. The character of carbohydrate–aromatic interactions is still a subject of controversy;[9,10] thus, the studies with suitable model systems provide important insights on the origin of the carbohydrate–aromatic interactions.

To compare the binding properties of receptors **1** and **2** with the properties of previously published receptors (for example, receptors **7** and **8**, see Fig. 5), the dodecyl β-D-maltoside (**3**), dodecyl α-D-maltoside (**4**), octyl β-D-glucopyranoside (**5**) and octyl α-D-glucopyranoside (**6**) were selected as substrates. The interactions of the receptors and carbohydrates were investigated by ^{1}H NMR and fluorescence spectroscopy in organic media.[11–13] The ^{1}H NMR binding titration data were analyzed using the Hostest 5.6 program.[14] The fluorescence binding titration data were analyzed using the Hyperquad 2006 program.[15] Stoichiometry of the receptor–sugar complexes was determined by mole ratio plots and by the curve-fitting analysis of the titration data.

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† Electronic supplementary information (ESI) available: Description of the ^{1}H NMR and fluorescence titration experiments (Tables S1–S11). See DOI: 10.1039/b719212f

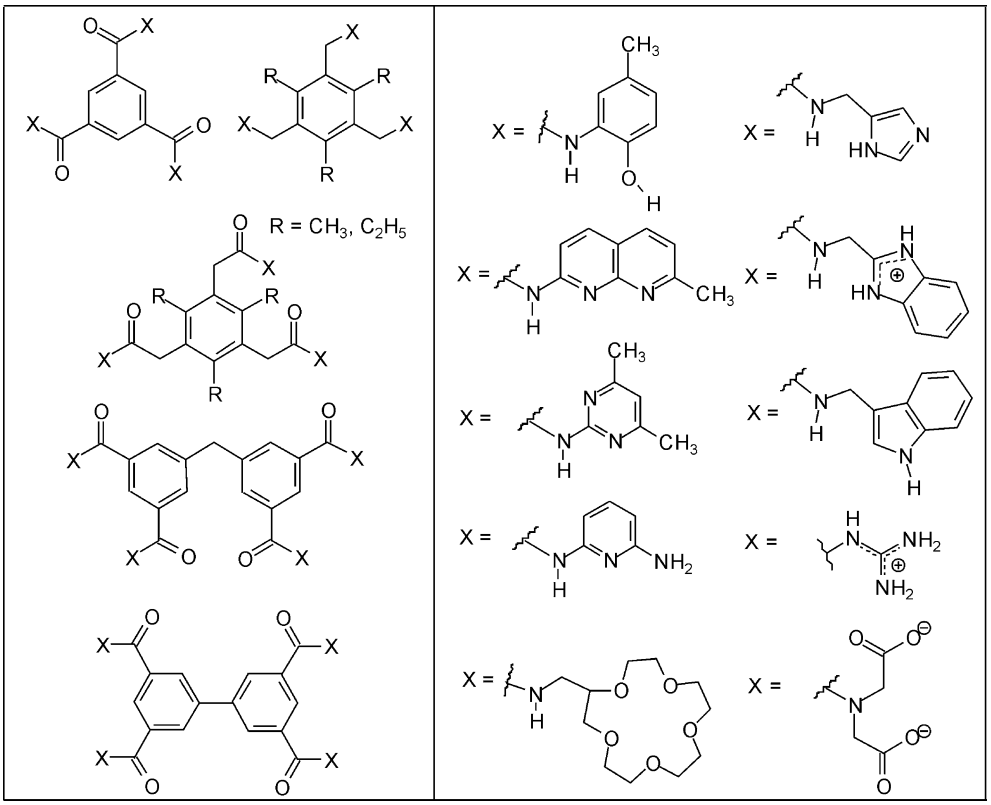


Fig. 2 Examples of spacer and recognition units used by our group for the construction of acyclic carbohydrate receptors.[5]

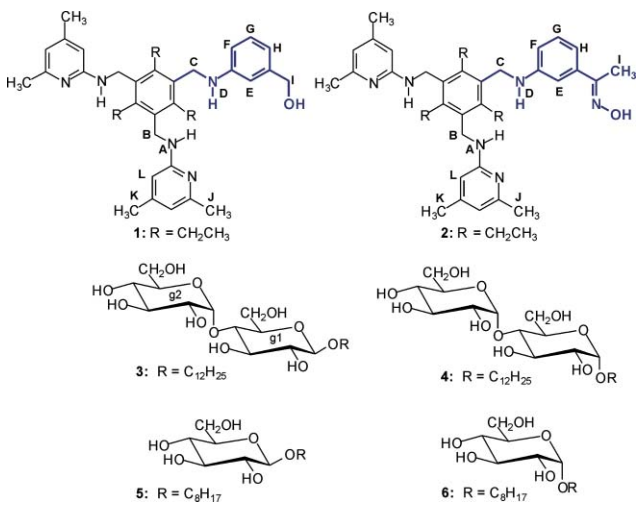


Fig. 3 Structures of receptors and sugars investigated in this study.

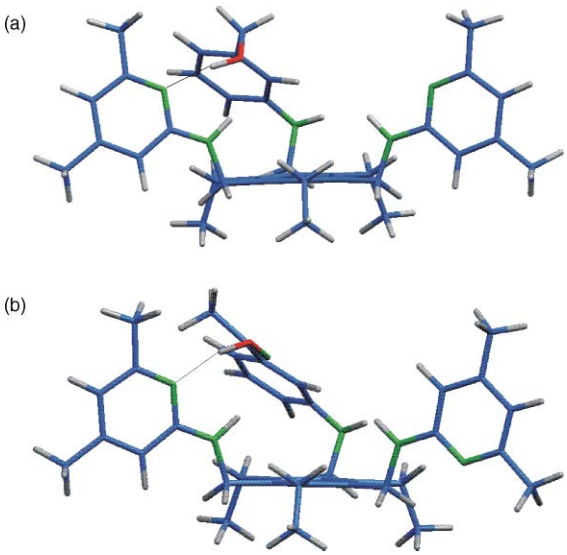


Fig. 4 Energy-minimized structure of the receptor **1** (a) and **2** (b). MacroModel V.8.5, OPLS-AA force field, MCMM, 25 000 steps. Color code: C, blue; N, green; O, red.

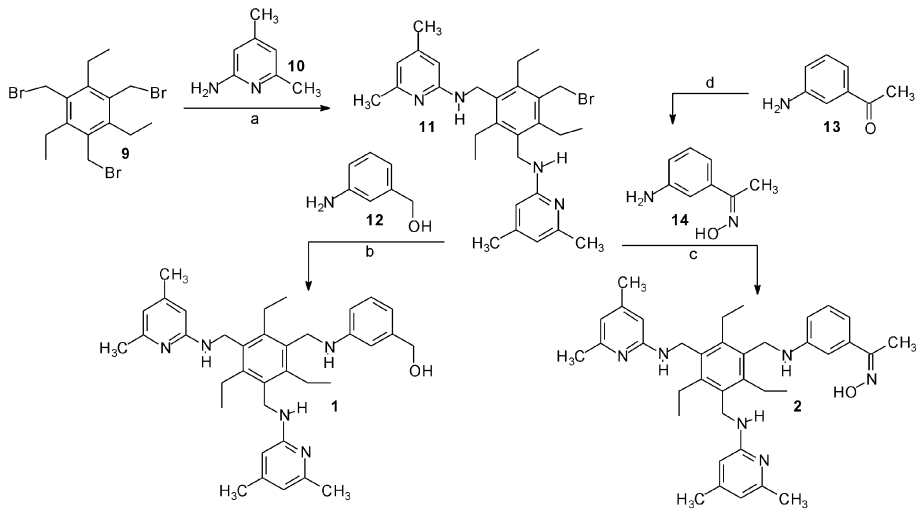
Results and discussion

Synthesis of the receptors

The base for the synthesis of compounds **1** and **2** was the compound **11**, which was prepared *via* a reaction of 1,3,5-tris(bromomethyl)-2,4,6-triethyl-benzene[16] (**9**) with 2 equivalents of 2-amino-4,6-dimethylpyridine (**10**).[5a] The reaction of 1-bromomethyl-3,5-bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (**11**) with 3-aminobenzylalcohol (**12**) or 3-aminobenzophenone oxime (**14**) provided the compounds **1** and **2**, respectively (see Scheme 1).

Binding studies with disaccharides 3 and 4

β- and α-maltoside, **3** and **4**, are poorly soluble in $CDCl_3$, but could be solubilized in this solvent in the presence of the receptor **1** or **2**, indicating favourable interactions between the binding



Scheme 1 *Reaction conditions:* (a) 2 equiv. of **10**, CH_3CN–THF, K_2CO_3, 48 h (30%); (b) CH_3CN–THF, K_2CO_3, 72 h (73%); (c) CH_3CN–THF, K_2CO_3, 72 h (61%); (d) $NH_2OH \times HCl$, NaOH, CH_3CH_2OH–H_2O.

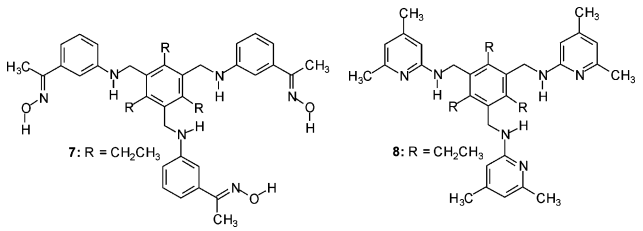


Fig. 5 Structures of the previously studied symmetrical receptors **7** and **8**.[5c,5k]

partners (similar solubility behaviour of the disaccharides **3** and **4** was observed in the presence of the previously described three-armed oxime-based receptors[5c]). Thus, the receptor in $CDCl_3$ was titrated with a solution of maltoside dissolved in the same receptor solution. The complexation between **1** or **2** and both disaccharides was evidenced by several changes in the NMR spectra (for examples, see Fig. 6 and 7).

During the titrations of **1** with **3** or **4** the signal due to the amine NH^A of **1** moved downfield by about 0.60 and 0.90 ppm, respectively; the addition of 0.5 equiv of sugar **3** or **4** led to practically complete complexation of **1**. The NH^D signal shifted downfield with strong broadening (by about 0.4 and 0.8 ppm, respectively); this signal was overlapping during the titration with **3** or **4**, and could not be used for the determination of the binding constants. Furthermore, the ^1H NMR spectra showed changes in the chemical shifts of the CH_3, CH_2 and CH resonances of **1** (up- and downfield shifts in the range of 0.03–0.10 ppm; see Fig. 6 and Table 1). The shifts of the NH^A, CH_2, CH_3 and aromatic CH protons of **1** were monitored as a function of sugar concentration; typical titration plots are shown in Fig. 8. The mole ratio plots indicated the formation of complexes with 2 : 1 receptor–sugar binding stoichiometry. The best fit of the titration data for **1•3** and **1•4** was obtained with the 2 : 1 receptor–sugar binding model;[14,17] however, the binding constants were too large to be accurately determined by ^1H NMR titrations[18] (see Table 1).

Table 1 Association constants[a–d] for receptors **1** and **2** and carbohydrates **3–6**

Host–guest complex	K_{11}/M^{-1}	K_{21}[e] or K_{12}[f]/M^{-1}	$\beta_{21} = K_{11}K_{21}$ or $\beta_{12} = K_{11}K_{12}$/M^{-2}	$\Delta\delta_{obs}$[g]/ppm
1•3		>100 000[b,e]		NH^A: 0.60; CH_2^B: −0.09; NH^D: 0.80; CH^E: 0.09; CH^H: −0.08; CH_3^J: −0.03
1•4		>100 000[b,e]		NH^A: 0.90; CH_2^B: −0.07; NH^D: 0.40; CH^E: 0.09; CH^H: −0.07; CH_3^J: −0.03
1•5	1830[b]	180[b,f]	3.29×10^5	NH^A: 1.31; CH_2^B: −0.15; CH^E: 0.14; CH^F: 0.11; CH_3^J: −0.06
2•3	371 200[c]	7950[c,e]	2.95×10^9	NOH: −1.70; CH^E: 0.16; CH^H: −0.10; CH^F: 0.08; CH_3^J: −0.05
2•4	187 930[c]	7010[c,e]	1.31×10^9	NOH: −1.44; CH^E: 0.20; CH^H: −0.08; CH^F: 0.10; CH_3^J: −0.06
2•5	2050[b]	720[b,f]	1.48×10^6	NOH: −2.36; NH^A: 0.89; CH_2^B: −0.10; CH_2^C: −0.16; CH^E: 0.26; CH^F: 0.15
2•6	790[c]	270[c,f]	2.13×10^5	

[a] Average K_a values from multiple titrations. [b] Determined on the base of ^1H NMR spectroscopic titrations in $CDCl_3$. [c] Determined on the base of fluorescence titrations in $CHCl_3$. [d] Errors in K_a are less than 10%. [e] K_{21} corresponds to a 2 : 1 receptor–sugar association constant. [f] K_{12} corresponds to a 1 : 2 receptor–sugar association constant. [g] Largest change in chemical shift observed during the ^1H NMR titrations for the receptor signals (the concentration of receptor was kept constant and that of sugar varied); down- and upfield (−) shifts.

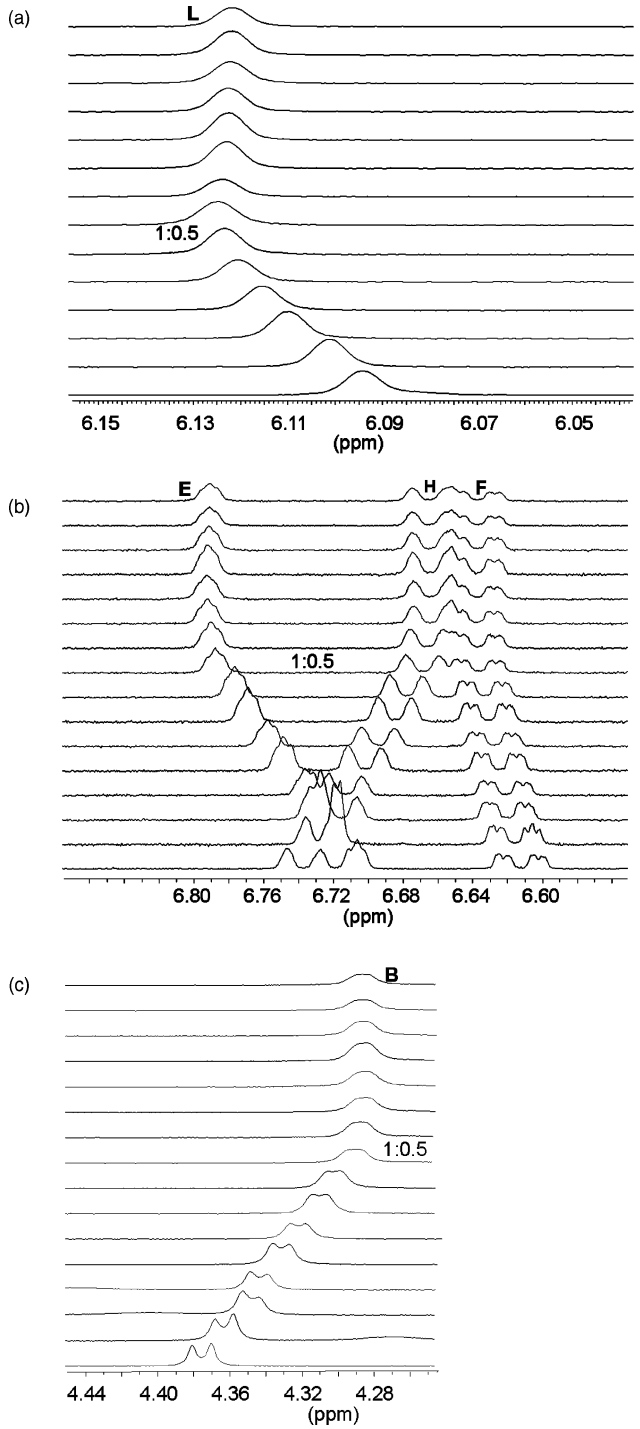


Fig. 6 (a–c) Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$) of **1** after addition of (from bottom to top) 0.00–3.13 equiv of β-maltoside **3** ([**1**] = 1.02 mM). Shown are chemical shifts of the pyridine CH^L resonances of **1** (for labeling, see formula **1**). (b, c) Partial ^{1}H NMR spectra of **1** after addition of (from bottom to top) 0.00–1.95 equiv of α-maltoside **4** ([**1**] = 1.01 mM). Shown are chemical shifts of the phenyl CH and CH_2 resonances of **1** (protons E, H, F, and B; for labeling, see formula **1**).

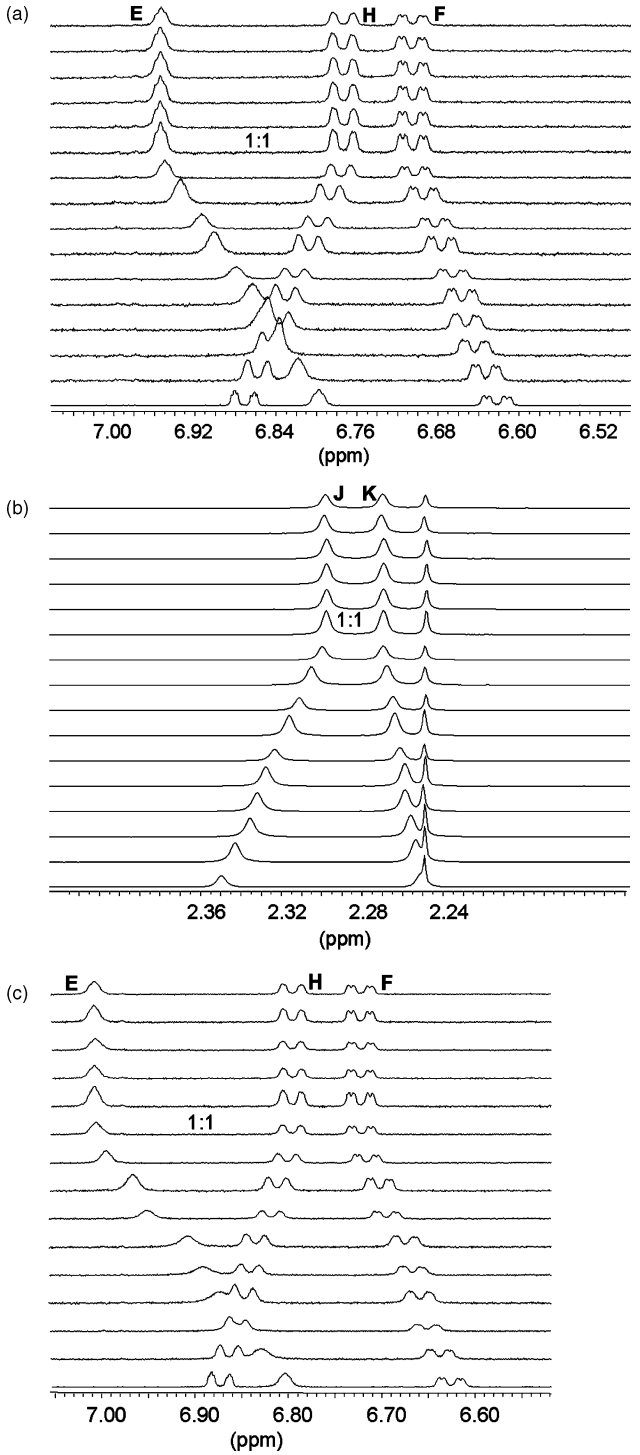


Fig. 7 (a, b) Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$) of **2** after addition of (from bottom to top) 0.00–3.06 equiv of β-maltoside **3** ([**2**] = 1.02 mM). Shown are chemical shifts of the $CH^{E,H,F}$ and CH_3 resonances of **2** (for labeling, see formula **2**). (c) Partial ^{1}H NMR spectra of **2** after addition of (from bottom to top) 0.00–3.09 equiv of α-maltoside **4** ([**2**] = 1.03 mM). Shown are chemical shifts of the $CH^{E,H,F}$ resonances of **2**.

The ^{1}H NMR spectra obtained during the titrations of **2** with the disaccharide **3** or **4** showed large shifting of the OH and NH^A resonances; however, the strong broadening of these resonances prevented their use in the estimation of the binding constants. The signal due to the NH^D of **2** was unobservable after the addition of only 0.1 equiv of the disaccharide **3** or **4**. The signal due to the oxime OH of **2** moved upfield with strong broadening by 1.70 and 1.45 ppm after the addition of **3** and **4**,

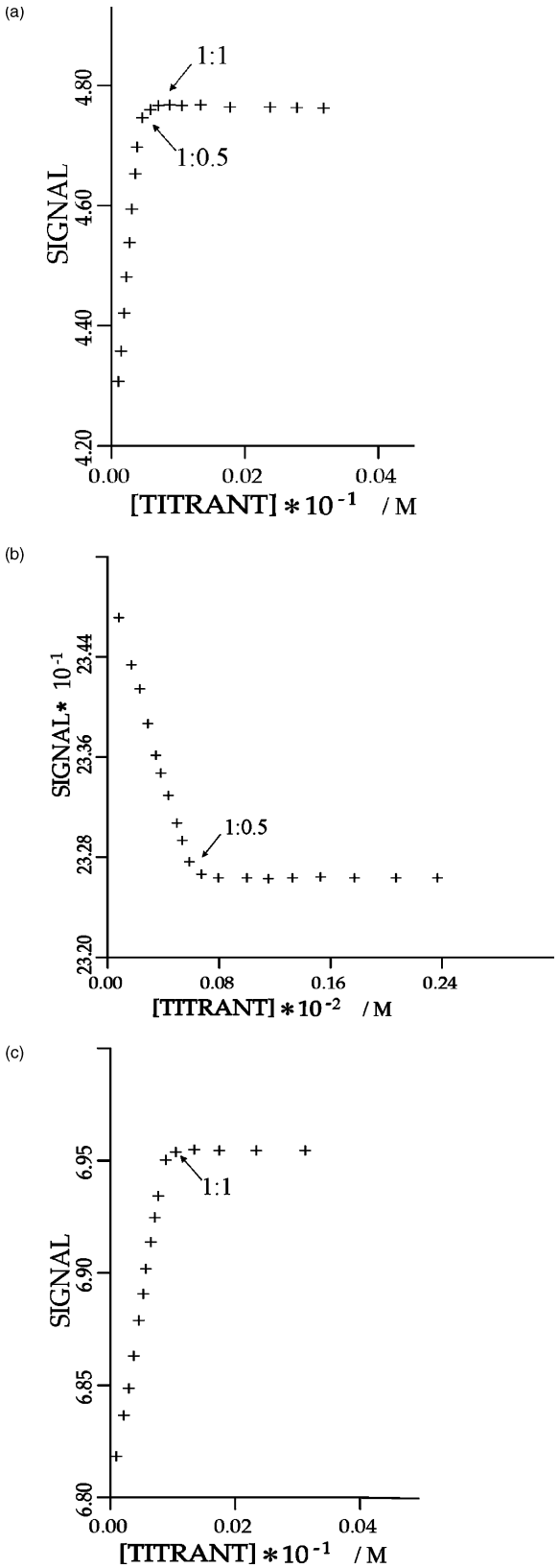


Fig. 8 (a and b) Plot of the observed chemical shifts of the NH^A and $CH_3{}^J$ resonances of **1** as a function of added β-maltoside **3** (a) or α-maltoside **4** (b). (c) Plot of the observed chemical shifts of the phenyl CH resonances of **2** as a function of added β-maltoside **3**. The [receptor] : [sugar] ratio is marked.

respectively. This signal was almost unobservable after the addition of about 0.1 equiv of the corresponding disaccharide, and became distinct near the saturation, that occurred after the addition of about 0.8 equiv of the disaccharide **3** or **4**. In the case of the receptor **2**, the molecular modeling calculations indicated the formation of an intramolecular hydrogen bond between the oxime OH and the pyridine nitrogen of **2**, as shown in Fig. 4a. The existence of the intramolecular hydrogen bond was confirmed by NMR spectroscopy. The resonance for the oxime OH proton of **2** in $CDCl_3$ solution was independent of its concentration and occurred at ~11.5 ppm (the resonance for the oxime OH protons of the previously described three-armed oxime-based receptors[5c] occurred at 7.5 ppm; 1 mM $CDCl_3$ solution).[19] The observed complexation-induced shifts of the OH and NH signals indicated important contribution of the OH and NH groups of **2** to the complex formation through formation of intermolecular hydrogen bonds with the disaccharide **3** or **4**.

The complexation between receptor **2** and the both disaccharides was further evidenced by up- and downfield chemical shifts of the CH_2, CH_3, pyridine CH and phenyl CH protons (in the range of 0.03–0.20 ppm; see Table 1 and Fig. 7). The fit of the NMR shift changes of these resonances agreed with a "mixed" 1 : 1 and 2 : 1 receptor–sugar binding model. The results of the ^{1}H NMR titrations indicated the formation of very strong 1 : 1 complexes ($K_{11} > 100\,000$ M^{-1}) and weaker complexes with 2 : 1 receptor–sugar stoichiometry (the binding constants were too large to be accurately determined by ^{1}H NMR titrations).

The formation of strong complexes between the receptor **2** and the disaccharide **3** or **4** was confirmed by fluorescence spectroscopy[20] (the binding properties of the receptor **1** could not be analysed on the base of fluorescence spectroscopy). The fluorescence titration experiments were carried out by adding increasing amounts of the sugar **3** or **4** (both disaccharides are soluble in $CHCl_3$ in the concentration range required for fluorescence titrations) to a $CHCl_3$ solution of the receptor **2** (for example, see Fig. 9a). The best fit of the titration data (at 406 nm) was obtained with a "mixed" 1 : 1 and 2 : 1 receptor–sugar binding model; this binding model was further supported by the mole ratio plots. The binding constants for **2•3** were found to be 371 200 (K_{11}) and 7950 (K_{21}) M^{-1} ($\beta_{21} = 2.95 \times 10^9$ M^{-2}), whereas those for **2•4** amounted to 187 930 (K_{11}) and 7010 (K_{21}) M^{-1} ($\beta_{21} = 1.31 \times 10^9$ M^{-2}).[17c] Thus, the receptor **2** exhibits about 2-fold higher binding affinity toward the β-maltoside **3**.

According to molecular modeling calculations (see Fig. 10) the receptor–maltoside complexes are stabilized by hydrogen bonds between the OH groups as well as the ring-O of the sugar and the NH^A, NH^D, pyridine-N, CH_2OH (in the case of **1**) and oxime-OH/oxime-N (in the case of **2**) of the receptor **1** or **2** (the participation of the NH and OH groups of the receptors in the formation of the intermolecular hydrogen bonds with the sugar was confirmed by ^{1}H NMR spectroscopy; see above). The sugar OH groups are involved in cooperative and bidentate hydrogen bonds, similar to interactions in protein–carbohydrate complexes. Furthermore, CH···O/N hydrogen bonds and interactions of sugar CHs with the phenyl groups of the receptor molecule provide an additional stabilisation of the receptor–sugar complex. Examples of noncovalent interactions indicated by molecular modeling calculations for the complexes formed between receptor **1** or **2** and the disaccharide **3** are given in Table 2.

Table 2 Examples of noncovalent interactions indicated by molecular modeling calculations[a] for the complexes formed between receptor **1** or **2** and sugar **3**

Receptor–substrate complex	Noncovalent interactions[b,c]
1•3	(I) pyridine-N · · · HO-3 (g1); (I) NH^A · · · OH-2 (g1); (I) CH_2OH · · · OH-6 (g1);
2 : 1 receptor–sugar complex[b]	(II) pyridine-N · · · HO-6 (g1); (II) NH^A · · · O-ring (g1); (II) NH^A · · · OH-3 (g1);
	(II) NH^D · · · OH-2 (g1); (II) pyridine-N · · · HO-6 (g2); (I) NH^D · · · OH-2 (g2)
	(I) phenyl (central ring) · · · HC-1 (g1); (I) phenyl (central ring) · · · HC-3 (g1)
	(I) phenyl (central ring) · · · HC-5 (g1); (II) phenyl (central ring) · · · HC-2 (g1)
	(II) phenyl (central ring) · · · H_2C-6 (g1);
	(I) phenyl (hydroxymethyl-substituted) · · · HC-3 (g2)
	(II) CH_2OH · · · NH^A (I); (II) pyridine-CH_3 · · · N-pyridine (I)
2•3	NH^A · · · O-ring (g1); NH^A · · · OR; NH^D · · · OH-3 (g1); pyridine-N · · · HO-2 (g1)
1 : 1 receptor–sugar complex	pyridine-N · · · HO-6 (g1); CH_3^1 · · · OH-2 (g2); phenyl-CH · · · OH-3 (g1)
	phenyl (oxime-substituted) · · · HO-6 (g2); phenyl (oxime-substituted) · · · HC-1 (g2);
	phenyl (central ring) · · · HC-2 (g1); phenyl (central ring) · · · HC-4 (g1)
2•3	(I) NH^A · · · O-ring (g1); (I) NH^A · · · OH-2 (g1); (I) NH^D · · · OH-3 (g1);
2 : 1 receptor–sugar complex[b]	(I) pyridine-N · · · HO-2 (g1); (I) pyridine-N · · · HO-6 (g1); (I) phenyl-CH · · · OH-3 (g1)
	(II) NH^A · · · OH-2 (g2); (II) NH^A · · · OH-3 (g2); (II) NOH · · · OH-6 (g2);
	(II) pyridine-N · · · HO-4 (g2);
	(I) phenyl (central ring) · · · HC-2 (g1); (I) phenyl (central ring) · · · HC-4 (g1)
	(II) phenyl (oxime-substituted) · · · HC-1 (g1); (II) phenyl (oxime-substituted) · · · HC-3 (g1); (II) oxime-N · · · HC-5 (g1)

[a] MacroModel V.8.5, OPLS-AA force field, MCMM, 50 000 steps. [b] I and II: two receptors in the 2 : 1 receptor–sugar complex. [c] g1 and g2: the glucose units of **3** (for labeling see Fig. 3).

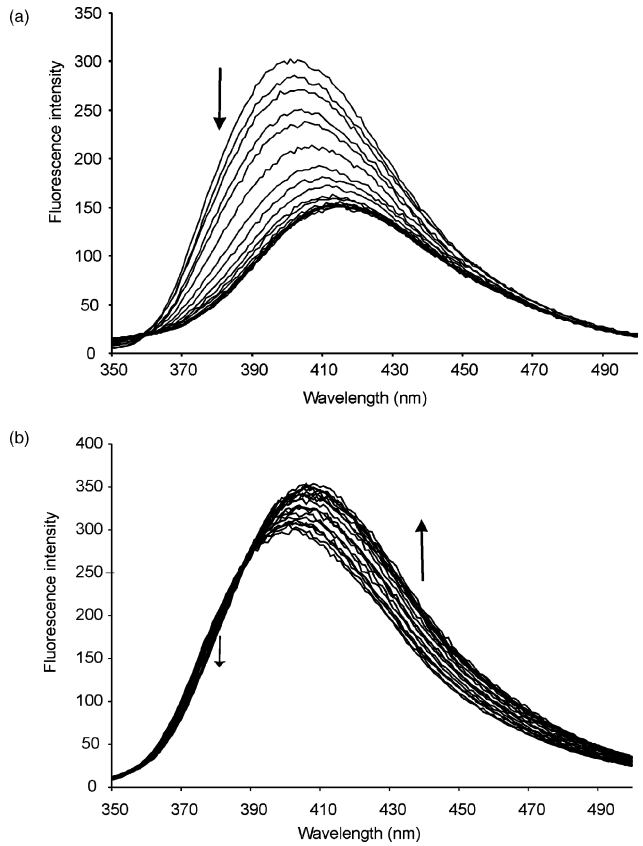


Fig. 9 Fluorescence titration of receptor **2** with β-maltoside **3** (a) and β-glucopyranoside **5** (b) in $CHCl_3$; [**2**] = 0.17 mM; Equiv of **3** = 0.00, 0.07, 0.15, 0.23, 0.31, 0.46, 0.62, 0.77, 0.93, 1.08, 1.24, 1.40, 1.55, 1.71, 1.86, 2.02, 2.17, 2.33, 2.48, and 2.64; Equiv of **5** = 0.00, 0.29, 0.58, 0.88, 1.17, 1.76, 2.35, 2.93, 3.52, 4.11, 4.70, 5.29, 5.87, 6.46, 7.05, 7.64, 8.23, 8.81, 9.40, and 9.99. Excitation wavelength 336 nm.

Binding studies with monosaccharides 5 and 6

The ^{1}H NMR titration experiments with β-glucopyranoside **5** were carried out by adding increasing amounts of the sugar to a $CDCl_3$ solution of the receptor **1** or **2**. Similar to the binding studies with disaccharide **3** or **4**, the complexation between **1** or **2** and glucopyranoside **5** was evidenced by several changes in the NMR spectra (for examples, see Fig. 11). However, whereas after the addition of about 0.5 equiv (in the case of receptor **1**) or 1 equiv (in the case of receptor **2**) of the disaccharide **3** or **4** almost no more change was observed in the chemical shift of the receptor signals, with the monosaccharide **5**, chemical shift changes continued to higher [sugar] : [receptor] ratios. During the titrations of **1** or **2** with **5** the signal due to the amine NH^A of **1** moved downfield by about 1.3 ppm (after the addition of 8 equiv of sugar), whereas the NH^A of **2** shifted downfield by 0.9 ppm. The signal due to the oxime OH of **2** shifted significantly upfield by about 2.3 ppm with broadening. Furthermore, the ^{1}H NMR spectra showed changes in the chemical shifts of the CH_3, CH_2, and phenyl CH's protons (up- or downfield shifts in the range of 0.04–0.26 ppm; see Table 1), as illustrated in Fig. 11. The curve fitting of the titration data suggested the existence of 1 : 1 and 1 : 2 receptor–monosaccharide complexes in the chloroform solution (typical titration curves are shown in Fig. 12), with a stronger association constant for the 1 : 1 binding and a weaker association constant for the 1 : 2 receptor–sugar complex. The binding constants for **1•5** were found to be 1830 (K_{11}) and 180 (K_{12}) M^{-1} ($\beta_{12} = 3.29 \times 10^5$ M^{-2}), whereas those for **2•5** amounted to 2050 (K_{11}) and 720 (K_{12}) M^{-1} ($\beta_{12} = 1.48 \times 10^6$ M^{-2}).[17c]

Interactions between receptor **2** and β-glucopyranoside **5** could also be detected by fluorescence; however, the spectral changes observed during the fluorescence titrations with glucopyranoside **5** were less substantial than those observed during the titrations with disaccharides **3** and **4** (for example, see Fig. 9b). The analysis

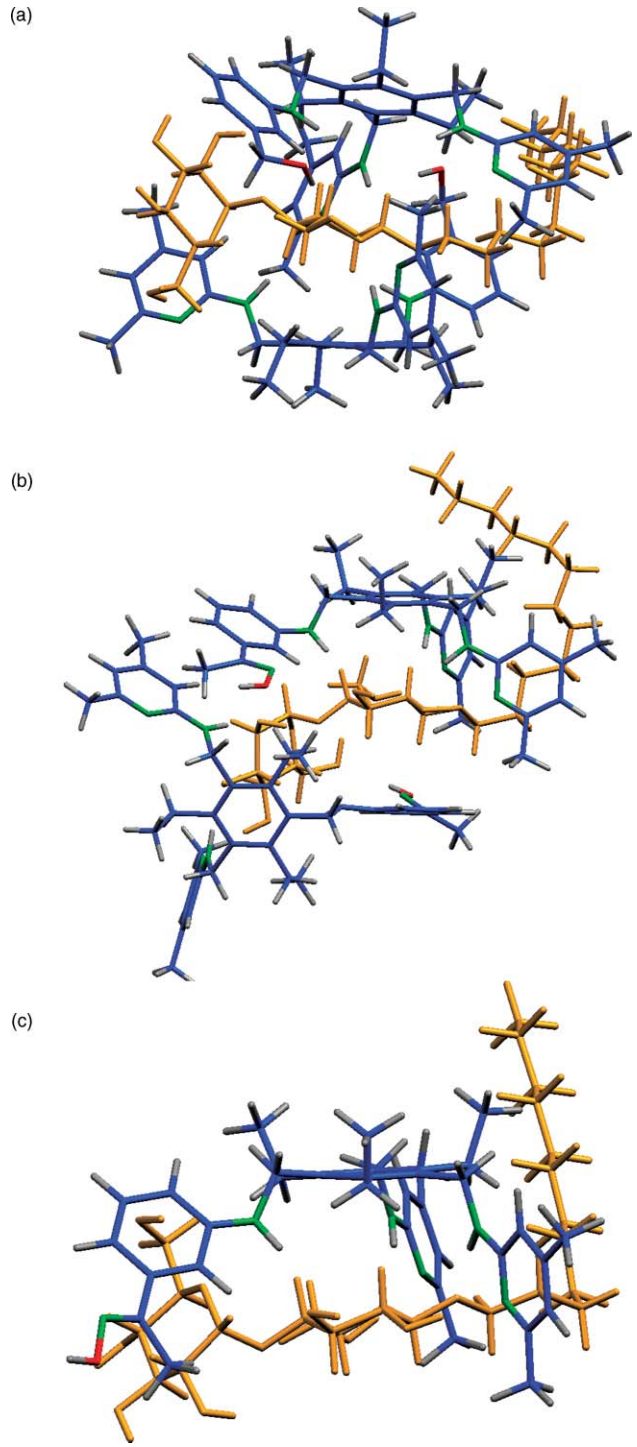


Fig. 10 (a) Energy-minimized structure of the 2 : 1 receptor–sugar complex formed between receptor **1** and β-maltoside **3**. (b and c) Energy-minimized structure of the 2 : 1 and 1 : 1 receptor–sugar complex formed between receptor **2** and β-maltoside **3**. MacroModel V.8.5, OPLS-AA force field, MCMM, 50 000 steps. Color code: receptor C, blue; receptor N, green; receptor O, red; the sugar molecule is highlighted in orange.

of the titration data confirmed the "mixed" 1 : 1 and 1 : 2 receptor–glucopyranoside binding model; the binding constants determined on the base of fluorescence titrations in $CHCl_3$ were comparable

Table 3 Association constants[a,b] for receptors **7** and **8** and carbohydrates **3–5**

Host–guest complex	K_{11}/M^{-1}	K_{21}[e] or K_{12}[f]$/M^{-1}$	$\beta_{21} = K_{11}K_{21}$ or $\beta_{12} = K_{11}K_{12}/M^{-2}$
7•3	100 500[c,g]		
	98 900[d,g]		
7•4	65 300[c,g]		
	62 000[d,g]		
7•5	170[c,g]	1730[c,f]	2.94×10^5
8•3	130 700[d]	42 300[d,e]	5.52×10^9
8•4	79 100[d]	16 350[d,e]	1.29×10^9
8•5	48 630[c,h]	1320[c,f]	6.42×10^7
	54 920[d]	1470[d,f]	8.07×10^7

[a] Average K_a values from multiple titrations. [b] Errors in K_a are less than 10%. [c] Determined on the base of ^{1}H NMR spectroscopic titrations in $CDCl_3$. [d] Determined on the base of fluorescence titrations in $CHCl_3$. [e] K_{21} corresponds to a 2 : 1 receptor–sugar association constant. [f] K_{12} corresponds to a 1 : 2 receptor–sugar association constant. [g] Results from ref. 5*c*. [h] Results from ref. 5*k*.

with those determined on the base of the NMR spectroscopic titrations in $CDCl_3$.

Fluorescence titrations of the receptor **2** with α-glucopyranoside **6** (fluorescence intensity increased with increasing monosaccharide concentration) indicated also the formation of complexes with 1 : 1 and 1 : 2 receptor–monosaccharide binding stoichiometry. The binding constants for **2•6** were found to be 790 (K_{11}) and 270 (K_{12}) M^{-1} ($\beta_{12} = 2.13 \times 10^5$ M^{-2}). Thus, the complexes formed between the receptor **1** or **2** and the monosaccharides **5** and **6** are much less stable than those formed with the disaccharides **3** and **4**.

Both the ^{1}H NMR and the fluorescence spectroscopic titrations clearly show the di- *vs.* monosaccharide preference of the receptors **1** and **2**. It should be noted that oligosaccharides have received far less attention in the artificial receptor chemistry than the monosaccharides,[21–24] and the selective recognition of oligosaccharides by receptors using noncovalent interactions is still rare.[21]

The comparison of the binding properties of the aminopyridine/oxime-based receptor **2** and the symmetrical oxime-based receptor **7** (see Table 1 and 3) shows that combining oxime- and aminopyridine-based recognition units significantly affect the binding properties. Both receptors, **2** and **7**, show strong di- *vs.* monosaccharide preference; however, receptor **2** exhibits higher affinity toward the tested disaccharides. In the case of receptor **7**, both glucose units of the disaccharide **3** or **4** have the possibility to interact with four phenyl rings of the receptor **7**; these interactions seem to be responsible for the 1 : 1 binding stoichiometry, similar to the complex between maltose binding protein (MBP) and maltose.[1h] Quiocho *et al.* pointed out that "the maltose is wedged between four aromatic side chains and the resulting stacking of these aromatic residues on the faces of the glucosyl units provides a majority of the van der Waals contacts in the complex."[1h]

The symmetrical pyridine-based receptor **8** has been established as highly effective receptor for both β-glucopyranoside **5** and maltosides **3**/**4**(see Table 3 and Fig. 13). The preference for the disaccharides is still observable, but is not so strong as in the case of **1**, **2** or **7** (for comparison, see Table 1 and 3).

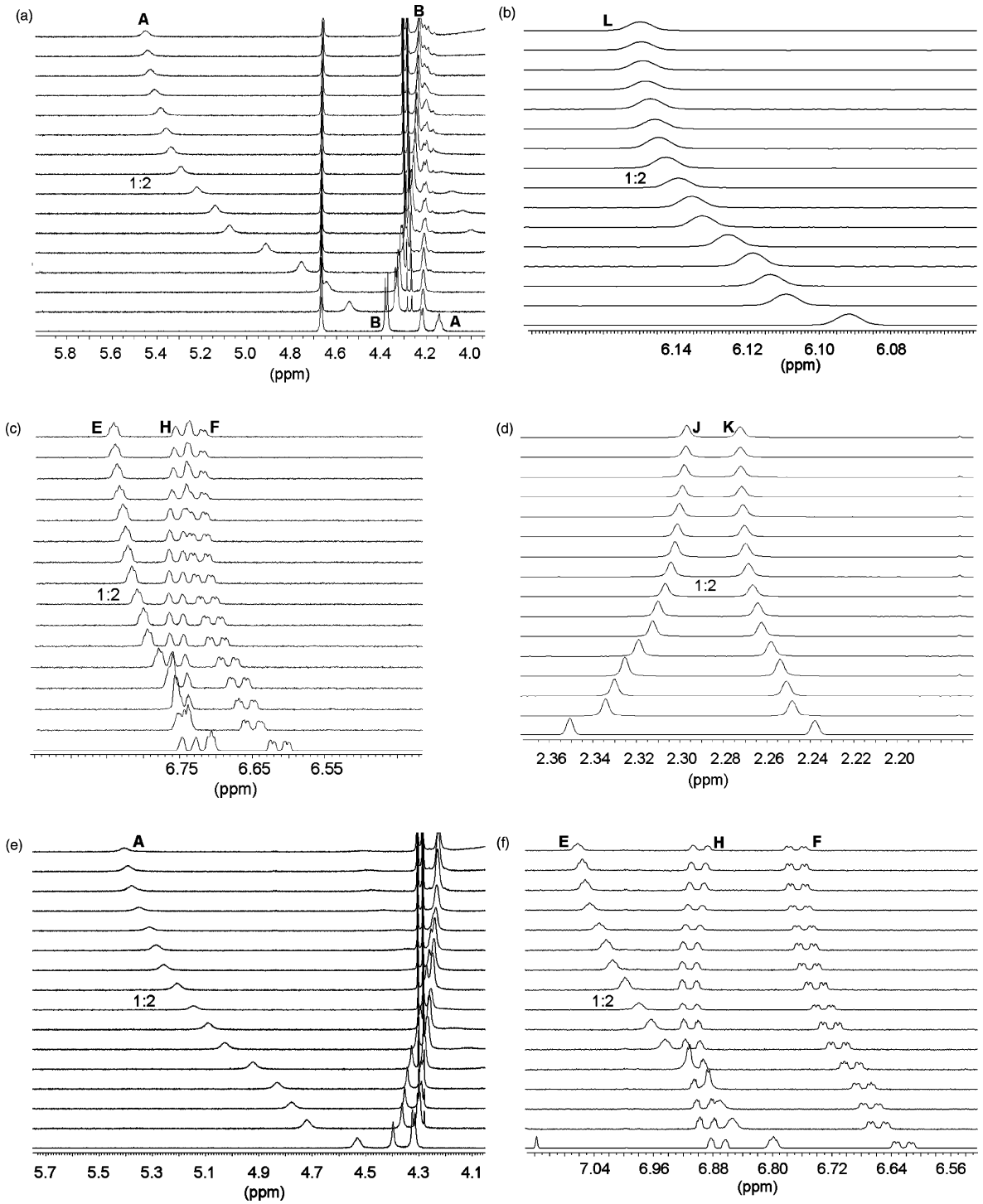


Fig. 11 (a–d) Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$) of **1** after addition of (from bottom to top) 0.00–8.02 equiv of **5** ([**1**] = 1.02 mM); for labeling, see formula **1**. (e, f) Partial ^{1}H NMR spectra of **2** after addition of (from bottom to top) 0.00–8.03 equiv of **5** ([**2**] = 1.02 mM); for labeling, see formula **2**.

Conclusion

Acyclic receptors **1** and **2** containing neutral hydrogen bonding sites, such as amine, pyridine, hydroxymethyl or oxime groups, were prepared, and their binding properties towards neutral sugar molecules studied. The two compounds have been established as highly effective receptors for β- and α-maltoside, **3** and **4**; the complexes formed with these disaccharides are much more stable than those formed with the monosaccharides **5** and **6** (see Table 1).

The formation of receptor–sugar complexes has been characterized by ^{1}H NMR spectroscopy and confirmed by a second, independent technique, namely fluorescence spectroscopy (in the case of the receptor **2**). Receptor **1** has the tendency to form strong 2 : 1 receptor–sugar complexes with β- and α-maltoside (see Table 1). Both hydrogen-bonding and interactions of the

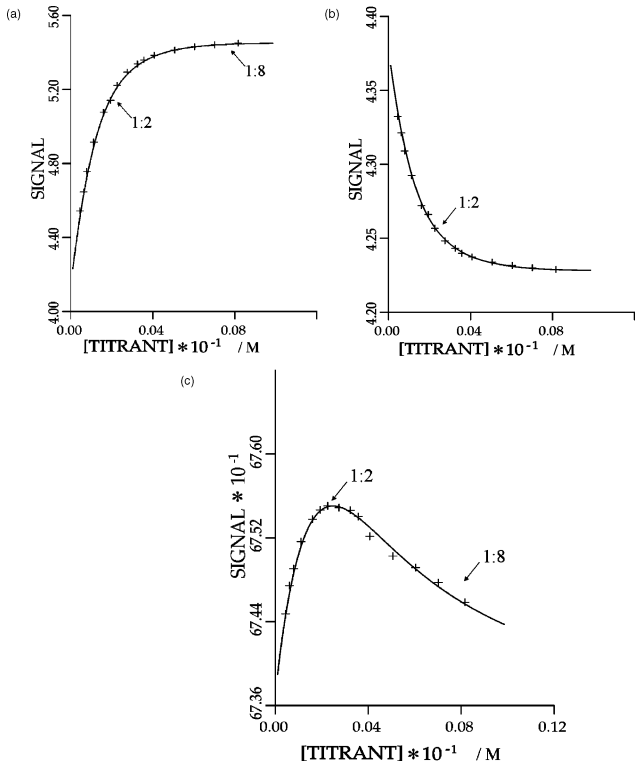


Fig. 12 Plot of the observed (+) and calculated (—) chemical shifts of the NH^A (a), $CH_2{}^B$ (b), and CH^H (c) resonances of **1** (1.02 mM) as a function of added β-glucopyranoside **5**. The [receptor] : [sugar] ratio is marked.

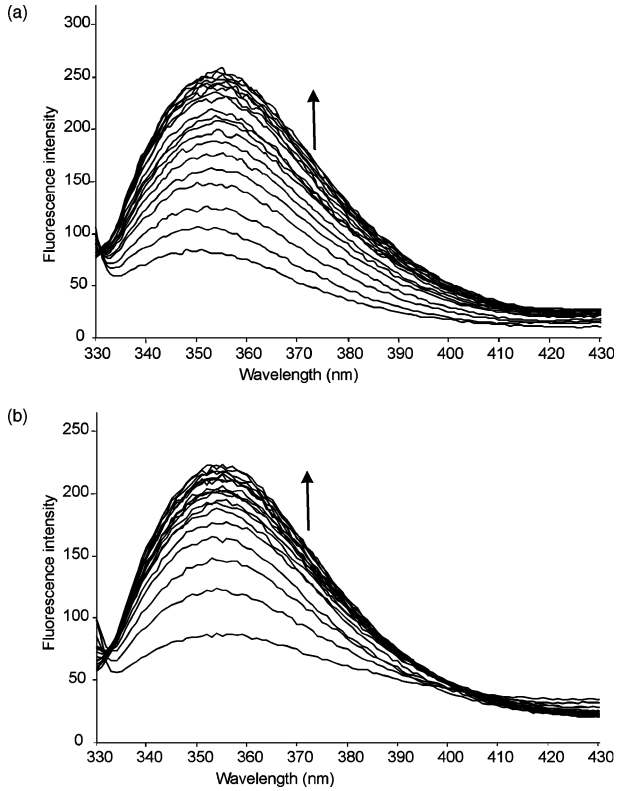


Fig. 13 Fluorescence titration of receptor **8** with β-maltoside **3** (a) and β-glucopyranoside **5** (b) in $CHCl_3$; [**8**] = 0.23 mM; Equiv of **3** = 0.00, 0.07, 0.16, 0.25, 0.33, 0.42, 0.50, 0.59, 0.67, 0.75, 0.84, 1.01, 1.18, 1.34, 1.51, 1.68, 2.02, 2.36, 2.69, and 2.86; Equiv of **5** = 0.00, 0.29, 0.58, 0.88, 1.17, 1.76, 2.05, 2.35, 2.64, 2.93, 3.52, 4.11, 4.70, 5.28, 5.87, 7.05, 8.22, 9.40, and 9.89. Excitation wavelength 326 nm.

sugar CH's with the phenyl rings of the receptor contribute to the stabilisation of the receptor–sugar complexes (see Fig. 10a and Table 2). According to molecular modeling calculations, the disaccharide **3** is encapsulated in the cavity between the two receptor molecules in a similar way as in the protein–sugar complexes. Both glucose units of the disaccharide have the possibility to interact with four phenyl rings of the two receptor molecules (two central phenyl rings and two hydroxymethyl-subsituted phenyl rings; see Fig. 10a), similar to the complex between maltose binding protein and maltose.

The analysis of the titration data obtained on the base of ^{1}H NMR and fluorescence titrations of the receptor **2** with the disaccharide **3** or **4** indicated the existence of 1 : 1 and 2 : 1 receptor–maltoside complexes in the chloroform solutions, with a very high binding constant for the 1 : 1 complexes (see Table 1). Examples of the energy-minimized structure of the 1 : 1 and 2 : 1 receptor–maltoside complexes are illustrated in Fig. 10c and 10b, respectively. According to the molecular modeling calculations, the hydrogen bonding interactions are complemented by the CH-π interactions between the sugar CH's and the phenyl rings of the receptor **2**. The phenyl rings provides additional apolar contacts to a saccharide, similar to sugar-binding proteins, which commonly place aromatic surfaces against patches of sugar CH groups. The comparison of the binding properties of the receptor **2** and the previously described three-armed oxime-based receptors[5c] shows that combining oxime- and aminopyridine-based recognition units significantly affect the binding properties. As in natural complexes, the participation of different types of hydrogen-bonding groups in the recognition process is favorable for reaching high binding selectivity of the receptor.

The binding studies between both receptors and the monosaccharide **5** or **6** indicated the formation of complexes with 1 : 1 and 1 : 2 receptor–monosaccharide binding stoichiometry (with a higher binding constant for the 1 : 1 complex; see Table 1). The results of the NMR and fluorescence titrations clearly showed that the receptors **1** and **2** are able to recognize both mono- and disaccharides, with a strong preference for the disaccharides.

Synthetic receptors using noncovalent interactions for sugar binding provide valuable model systems to study the basic molecular features of carbohydrate recognition. In this context, the acyclic receptors represent particularly interesting objects for systematic studies toward recognition motifs for carbohydrates.

Experimental

Analytical TLC was carried out on silica gel 60 F_{254} plates. Melting points are uncorrected. Dodecyl β-D-maltoside (**3**), dodecyl α-D-maltoside (**4**), octyl β-D-glucopyranoside (**5**), 3-aminobenzylalcohol (**12**), and 3-aminobenzophenone (**13**) are commercially available.

1-[(3-Hydroxymethyl-phenyl)aminomethyl]-3,5-bis-[(4,6-dimethylpyridin-2-yl)amino-methyl]-2,4,6-triethylbenzene (1)

A mixture of 1-bromomethyl-3,5-bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (**11**) (0.50 g, 0.96 mmol), 3-aminobenzyl alcohol (**12**) (0.153 g, 1.24 mmol) and K_2CO_3

(1 g, 7 mmol) in THF–CH_3CN (160 mL, 1 : 1 v/v) was stirred at room temperature for 72 h (the solution was monitored by TLC). After filtration and evaporation of solvents, the crude product was purified by column chromatography (aluminium oxide; chloroform–diethyl ether, 1 : 6 v/v). Yield 73%. M.p. 74–75 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.24 (m, 9 H), 2.24 (s, 6 H), 2.34 (s, 6 H), 2.75 (m, 6 H), 3.51 (br. s, 1 H), 4.14 (br. s, 2 H), 4.21 (d, J = 4.20 Hz, 2 H), 4.37 (d, J = 4.20 Hz, 4 H), 4.65 (s, 2 H), 6.09 (s, 2 H), 6.35 (s, 2 H), 6.60 (m, 1 H), 6.71 (m, 2 H), 7.19 (t, J = 7.80 Hz, 1 H). ^{13}C-NMR: δ = 16.85, 16.90, 21.11, 22.84, 22.91, 24.15, 40.68, 42.22, 65.53, 103.34, 110.63, 111.98, 113.98, 115.89, 129.51, 133.07, 133.20, 142.31, 143.60, 143.68, 148.46, 148.88, 156.74, 158.22. HR-MS (EI) calcd for $C_{36}H_{47}N_5O$: 565.3780; found: 565.3781. R_f = 0.54 (methanol–chloroform 1 : 7, v/v).

1-[(3-Acetyl-phenyl)aminomethyl]-3,5-bis-[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene oxime (2)

A mixture of 1-bromomethyl-3,5-bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (**11**) (0.42 g, 0.80 mmol), 3-aminobenzophenone oxime (**14**) (0.157 g, 1.05 mmol) and K_2CO_3 (1 g, 7 mmol) in CH_3CN–THF (80 mL, 1 : 1, v/v) was stirred at room temperature for 72 h (the solution was monitored by TLC). After filtration and evaporation of solvents, the crude product was purified by column chromatography (aluminium oxide; chloroform–diethyl ether, 4 : 6 v/v). Yield 61%. M.p. 71–72 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.18 (t, J = 7.6 Hz, 6 H), 1.24 (t, J = 7.6 Hz, 3 H), 2.24 (s, 3 H), 2.25 (s, 6 H), 2.36 (s, 6 H), 2.73 (q, J = 7.6 Hz, 2 H), 2.90 (q, J = 7.6 Hz, 4 H), 3.87 (br. s, 1H), 4.32 (d, J = 4.1 Hz, 4 H), 4.39 (s, 2 H), 4.52 (br. s, 2H), 6.11 (s, 2 H), 6.34 (s, 2 H), 6.60 (m, 1 H), 6.79 (m, 1 H), 6.87 (m, 1H), 7.13 (t, J = 7.8 Hz, 1 H), 11.48 (s, 1 H). ^{13}C-NMR: δ = 12.60, 16.56, 16.74, 21.25, 23.01, 23.68, 40.80, 42.90, 103.13, 110.17, 113.88, 115.09, 116.21, 128.90, 132.73, 133.43, 138.20, 143.21, 143.66, 148.24, 149.37, 155.61, 156.37, 158.00. HR-MS calcd for $C_{37}H_{49}N_6O$ (ESI): 593.3974; found: 593.3974. R_f = 0.32 (chloroform–diethyl ether, 4 : 6).

Acknowledgements

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