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Amide, Amino, Hydroxy and Aminopyridine Groups as Building Blocks for Carbohydrate Receptors

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Receptors **1**–**3**, incorporating primary amide, hydroxy or amino groups as recognition units used in nature, as well as 2-aminopyridine units as heterocyclic analogues of the asparagine/glutamine primary amide side chains, were prepared and their binding properties towards neutral sugar molecules were studied. The design of these receptors was inspired by the binding motifs observed in the crystal structures of protein–carbohydrate complexes. The binding studies with β-glucopyranoside **5** indicated the formation of complexes with 1:1 and 2:1 receptor–monosaccharide binding stoichiometries, with an overall β_{21} binding constant of 10^7–10^8 M^{-2}. Both hydrogen bonding and interactions of the sugar CH groups with the central phenyl rings of the receptors contribute to the stabilisation of the receptor–sugar complexes. The syntheses, molecular modeling studies and binding properties of the receptors **1**–**3** are described, as well as comparative binding studies with receptor **4**, lacking the third recognition site.

Introduction

The design of artificial carbohydrate receptors operating through noncovalent interactions remains a subject of intensive current research.[1,2] Consideration of the crystal structures of protein–carbohydrate complexes[3] provides much of the inspiration for the development of such receptors. Our previous studies have shown that mimicking of the binding motifs observed in the crystal structures of protein–carbohydrate complexes, through the use of natural recognition groups or their analogues,[2e,2i,2q] represents a powerful strategy for the design of effective and selective carbohydrate receptors.

The aim of this work was to explore the potential of receptors **1**–**3** (Scheme 1) – each containing two different types of neutral hydrogen-bonding sites – in carbohydrate recognition; the design of these receptors was inspired by the binding motifs shown in Figure 1. Receptors **1**–**3** incorporate primary amide, hydroxy or amino groups as recognition units used in nature, as well as 2-aminopyridine units[4] as heterocyclic analogues of the asparagine/glutamine primary amide side chains.[5] As in natural complexes, the participation of different types of hydrogen-bonding groups in the recognition process was expected to be favourable for achieving high binding affinities and selectivities of the artificial receptors. Furthermore, the interactions between the central phenyl rings of the receptors and the sugar CH groups were expected to provide additional stabilization of the receptor–sugar complexes[6,7] (packing of an aromatic ring from the protein against sugars is observed in most carbohydrate-binding proteins[3]).

To compare the binding properties of receptors **1**–**3** with the properties of the previously published receptors, octyl β-D-glucopyranoside (**5**) and octyl α-D-glucopyranoside (**6**) were selected as substrates for binding studies in organic media. In addition, the binding properties of receptors **1**–**3** were compared with the properties of compound **4** (Figure 2), lacking the third hydrogen-bonding site. X-ray crystallographic data revealed that the hydrogen bonds between sugar-binding proteins and essential recognition determinants on sugars are shielded from bulk solvent, meaning that they exist in an environment with a lower dielectric constant[3] (see also ref.[8]). Thus, investigations with synthetic receptors in a medium with a lower dielectric constant, such as chloroform, make an important contribution to our understanding of the complex carbohydrate binding processes in nature.[9]

Results and Discussion

Synthesis of the Receptors

The basis for the synthesis of compounds **1**–**3** was compound **7** (see Scheme 1), prepared by treatment of 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene[10] with 2 equiv. of 2-amino-4,6-dimethylpyridine (see Exp. Sect.). The reaction of **7** with 1 equiv. of diethyl iminodiacetate provided the diester **8**, which was further transformed into the diamide **1** by treatment with methanolic ammonia. Treatment of **7** with aqueous ammonia gave the amino derivative **2**, while

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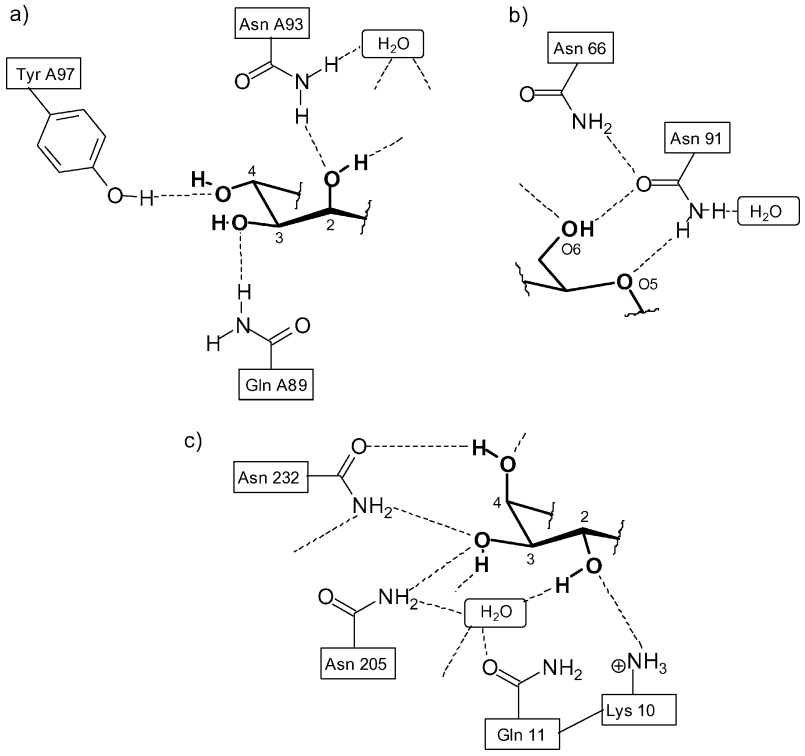


Figure 1. Examples of hydrogen bonds in the complexes of: a) *Galanthus nivalis* lectin with mannose,[3f,3a] b) D-galactose-binding protein with D-glucose,[3b] and c) L-arabinose-binding protein with L-arabinose.[3b]

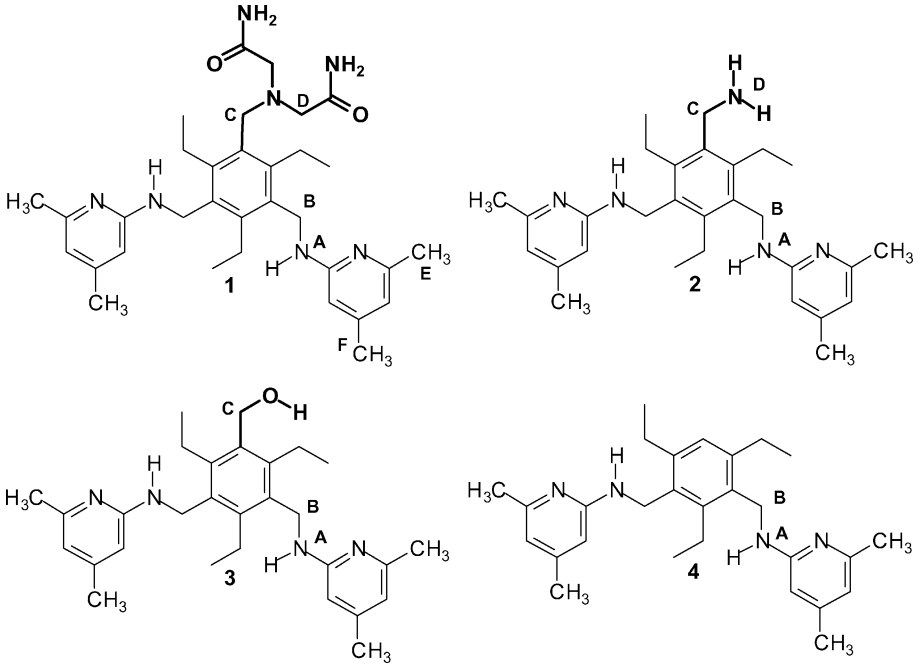
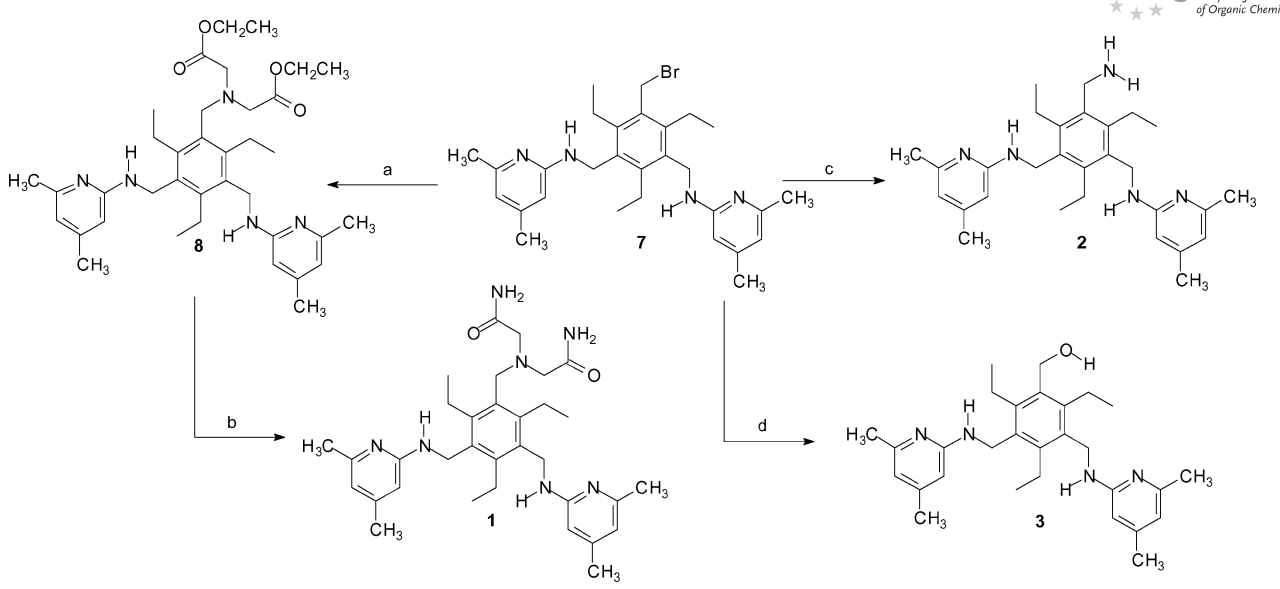


Figure 2. Structures of receptors **1**–**4**.

compound **3** was synthesized by treatment of **7** with sodium hydroxide (see Scheme 1 and Experimental Section). The synthesis of **4** involves the reaction of 1,3-bis(bromomethyl)-2,4,6-triethylbenzene (**9**)[10a] with 2 equiv. of 2-amino-4,6-dimethylpyridine.

Binding Studies

The interactions of the receptors **1**–**4** and carbohydrates **5** and **6** were investigated by ^{1}H NMR spectroscopy in [D_1]chloroform or in water-containing [D_1]chloroform. ^{1}H



Scheme 1. a) $HN(CH_2COOCH_2CH_3)_2$, THF/CH_3CN, K_2CO_3, 24 h, yield 60%; b) NH_3/CH_3OH, 48 h; yield 39%; c) NH_3/H_2O (25% solution), THF/CH_3OH, 12 h; yield 72%; d) NaOH aq, THF, 5 h; yield 75%.

NMR titration experiments[11] were carried out by addition of increasing amounts of the sugar to a $CDCl_3$ solution of the receptor. In addition, inverse titrations in which the concentration of sugar was held constant and that of the receptor was varied were performed. The ^1H NMR binding titration data were analysed by use of the Hostest 5.6 program[12] (stoichiometries of the receptor–sugar complexes were determined by mole ratio plots and by the curve-fitting analysis of the titration data).

Binding Properties of Receptor 1

During the titration of **1** with β-glucopyranoside **5** the signal due to the amide NH of **1** moved downfield by about 1.1 ppm (see Figure 3, a), with saturation occurring after the addition of about 1 equiv. of **5** (see Figure 4, a). The amine NH^A signal showed very strong broadening and was unobservable after the addition of only 0.1 equiv. of **5**. Furthermore, the ^1H NMR spectra showed changes in the chemical shifts of the CH_3 (protons E, F; for labelling, see Figure 2), CH_2 (protons B, C, D) and pyridine CH resonances of **1**. The signal for the protons B were moved upfield by 0.23 ppm with broadening and splitting (see Figure 3, b). The splitting of the CH_2^C signal of **1** was observed after the addition of about 0.1 equiv. of **5**, as shown in part a of Figure S1 (see Supporting Information), while the signal due to CH_2^D was shifted downfield by 0.10 ppm with splitting (see Figure S1, b). The signals due to the $CH_3^{E,F}$ (see Figure S1, c) and pyridine CH protons were shifted up- and downfield in ranges of 0.02–0.06 ppm. The amide NH, CH_2^B and CH_3^F signals were monitored for the determination of the binding constants; a typical titration curve is shown in Figure 4 (a). The best fit of the titration data was obtained with the "mixed" 1:1 and 2:1 receptor–sugar binding model; this model was further supported by mole ratio plots (see Figure S3a). The binding constants for **1**·**5** were found to be 144520 M^{-1} (K_{11}) and 4330 M^{-1} (K_{21}) ($\beta_{21} = 6.25 \times 10^8$ M^{-2}; Table 1).[11c] Similar complexation behaviour of β-glucopyranoside **5** could also be observed in water-containing $CDCl_3$ ([receptor]/[water] = 1:5).

In addition, the interactions between β-glucopyranoside **5** and the receptor **1** were investigated on the basis of inverse titrations in which the concentration of sugar **5** was held constant and that of receptor **1** was varied. During the titration of **5** with **1** the signals due to the OH protons of **5** were shifted downfield with strong broadening and were unobservable after the addition of about 0.1 equiv. of **1** (in the case of 6-OH, after the addition of about 0.5 equiv. of **1**), indicating important contribution of the OH groups of **5** to the complex formation. The complexation between **5** and the receptor **1** was further evidenced by chemical shift changes of the CH units of **5** (see Figure 3, c). The signals due to the 1-, 2-, 3- and 4-CH protons of **5** were shifted upfield by 0.06, 0.15, 0.20 and 0.25, respectively. The best fit of the titration data was obtained with the "mixed" 1:1 and 1:2 sugar–receptor binding model (see Figure 4, c); thus, the inverse titrations supported the existence of 1:1 and 2:1 receptor–sugar complexes in chloroform solution, with the stronger association constant for 1:1 binding and a weaker association constant for the 2:1 receptor–sugar complex. The association constants obtained on the base of these titrations are identical within the limits of uncertainty to those determined from titrations in which the roles of receptor and substrate were reversed.

Similarly to the binding studies between **1** and β-glucopyranoside **5**, complexation between **1** and the α anomer **6** was evidenced by several changes in the NMR spectra, as shown in Figure 3 (d) and Figure S2 (see Supporting Information). The signal due to the amide NH of **1** was moved downfield by about 1.1 ppm; the amine NH^A signal showed very strong broadening and was unobservable after the addition of about 1 equiv. of **5**. The CH_2^B and CH_2^D (for

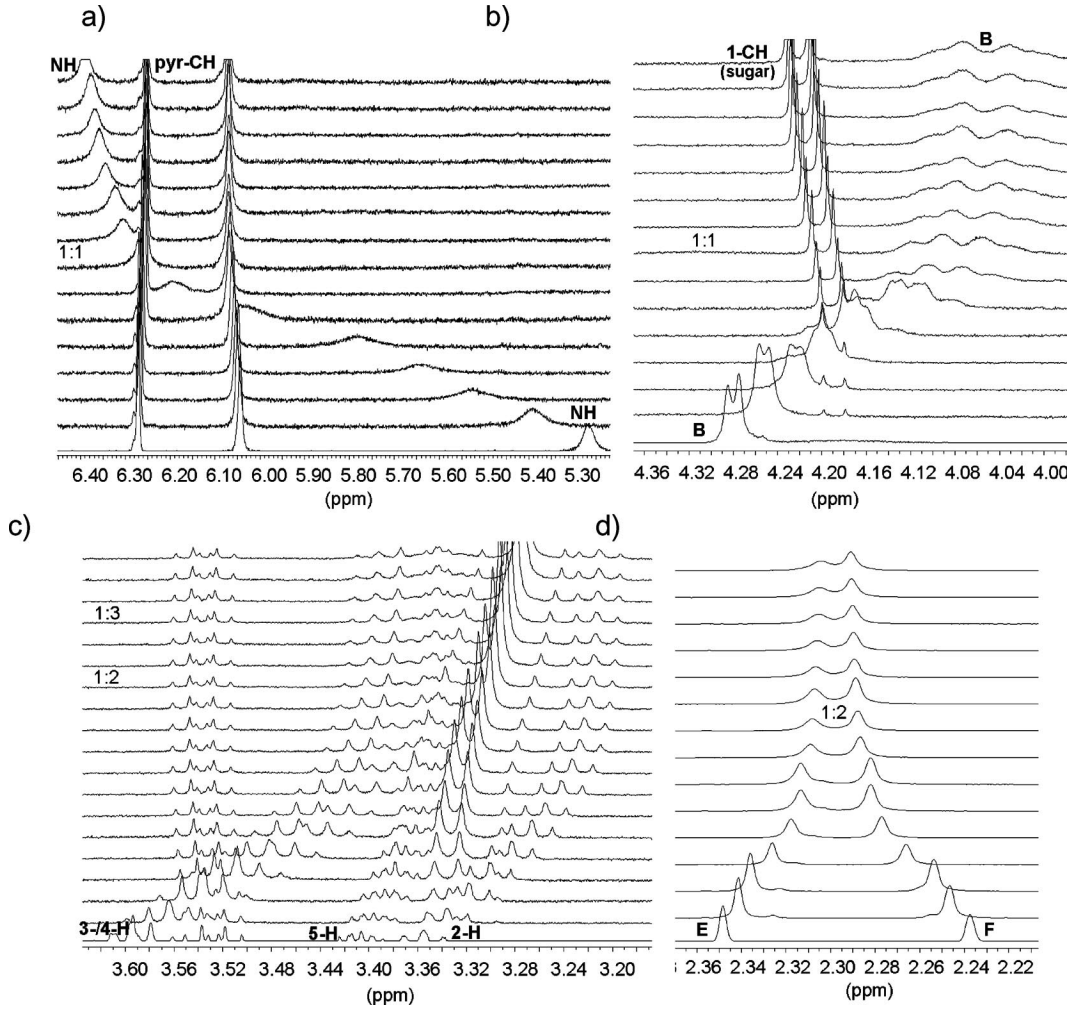


Figure 3. a, b) Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$) of **1** after addition of (from bottom to top) 0.00–3.03 equiv. of β-glucopyranoside **5** ([**1**] = 1.00 mM). Shown are chemical shifts of the amide NH and CH_2^B resonances of **1** (for labelling, see formula **1**). c) Partial ^{1}H NMR spectra (500 MHz. $CDCl_3$) of β-glucopyranoside **5** after addition of 0.00–4.39 equiv. of **1** ([**5**] = 0.68 mM). Shown are chemical shifts of the CH-2, -3, -4 and CH-5 resonances of **5** (as well as shifts of the OCH proton of **5**). d) Partial ^{1}H NMR spectra (400 MHz. $CDCl_3$) of receptor **1** after addition of 0.00–4.95 equiv. of α-glucopyranoside **6** ([**1**] = 0.85 mM). Shown are chemical shifts of the $CH_3^{E,F}$ resonances of **1**.

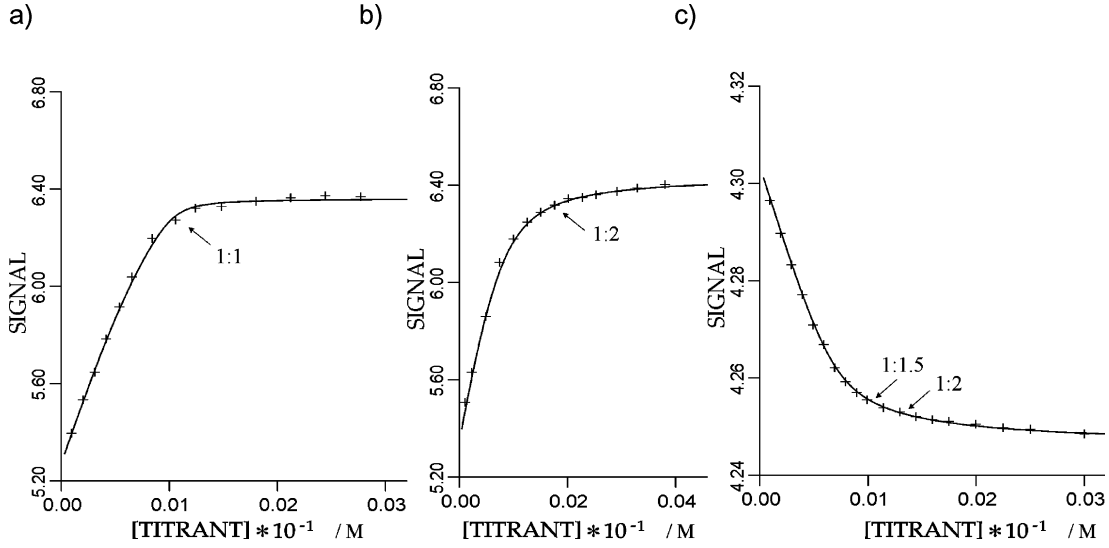


Figure 4. a, b) Plot of the observed (×) and calculated (–) chemical shifts of the amide NH signal of **1** (1.05 and 0.85 mM) as a function of added β-glucopyranoside **5** (a) or α-glucopyranoside **6** (b); the [receptor]:[sugar] ratio is marked. c) Chemical shift changes observed for 1-CH of **5** during the titration of **5** (0.68 mM) with **1** (inverse titration); the [sugar]:[receptor] ratio is marked.

Table 1. Association constants[a,b] for receptors **1**–**4** and carbohydrates **5** and **6**.

Receptor–substrate complex	K_{11} [M^{-1}]	K_{21}[c] or K_{12}[d] [M^{-1}]	$\beta_{21} = K_{11}K_{21}$ or $\beta_{12} = K_{11}K_{12}$ [M^{-2}]	$\Delta\delta_{obs}$[e] [ppm]
1·5	144520	4330[c]	6.25×10^8	amide-NH: 1.12; CH_2^B: –0.23; CH_3^F: 0.06; 1-CH: –0.06; 2-CH: –0.20; 4-CH: –0.25
1·6	24880	1750[d]	4.35×10^7	amide-NH: 1.06; CH_2^B: –0.14; CH_3^F: 0.04
2·5	39800	1610[c]	6.41×10^7	NH^A: 1.87; CH_2^B: –0.18; CH_3^E: –0.07; 1-CH: –0.23; 2-CH: –1.75
2·6	2280			NH^A: 1.21; CH_2^B: –0.13; CH_3^F: –0.05
3·5	18900	2850[c]	5.38×10^7	NH^A: 1.47; CH_2^B: –0.17; CH_3^E: –0.06; 1-CH: –0.19; 2-CH: –0.96
3·6	1840			NH^A: 1.22; CH_2^B: –0.14; CH_3^F: –0.05
4·5	1330			NH^A: 0.99; CH_2^B: –0.13; CH_3^E: –0.04

[a] Average K_a values from multiple titrations in $CDCl_3$. [b] Errors in K_a are less than 10%. [c] K_{21} corresponds to the 2:1 receptor–sugar association constant. [d] K_{12} corresponds to the 1:2 receptor–sugar association constant. [e] Largest change in chemical shift observed during the titration for NH, CH_2 and CH_3 signals of the receptor (down- and upfield shifts; the concentration of the receptor was kept constant and that of the sugar varied), as well as for the CH groups of the sugar in the case of inverse titrations (upfield shifts; the concentration of the sugar was kept constant and that of the receptor varied).

labelling, see Figure 2) signals were moved up- and downfield by 0.21 and 0.09 ppm, respectively (see parts a and c in Figure S2, Supporting Information). The splitting of the CH_2^B and CH_2^C signals of **1** was observed after the addition of only 0.1 equiv. of **5**, as shown in parts a and b of Figure S2 (see Supporting Information). In addition, the CH_3^E and CH_3^F signals were shifted up- and downfield by about 0.04 ppm (see Figure 3, d). Curve fitting of the titration data suggested the existence of 1:1 and 1:2 receptor–sugar complexes in the chloroform solution (different binding model from that determined for **1·5**); a typical titration curve is shown in Figure 4 (b). The binding constants for **1·6** were found to be 24880 M^{-1} (K_{11}) and 1750 M^{-1} (K_{21}) ($\beta_{21} = 4.35 \times 10^7$ M^{-2}). The binding studies with α-glucopyranoside **6** thus showed the interactions of receptor **1** with this monosaccharide to be less favourable than those with β-glucopyranoside **5**. However, the affinity of **1** toward the α anomer **6** is much higher than that of the previously described three-armed pyridine-based analogue {1,3,5-tris-[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene[4a]}.

Binding Properties of Receptors 2 and 3

The addition of β-glucopyranoside **5** to a solution of the receptor **2** caused a significant downfield shift of the amine NH^A signal of **2** (see Figure 5, b), as well as changes in the chemical shifts of the $CH_3^{E,F}$ (see Figure 5, a), $CH_2^{B,C}$ (see Figure S4) and pyridine CH resonances. The NH^A signal was moved downfield by about 1.9 ppm with broadening, whereas the NH_2 signal of **2** broadened during the titration and was unobservable after the addition of only 0.1 equiv. of **5**. The CH_2^B signal was shifted upfield by 0.18 ppm, while splitting of the CH_2^C signal was observed after the addition of 0.1 equiv. of **5**, as shown in Figure S4. Both the curve fitting of the titration data and the mole ratio plots suggested the existence of 1:1 and 2:1 receptor–sugar complexes in the chloroform solution, with a stronger association constant for 1:1 binding and a weaker association constant for the 2:1 receptor–sugar complex. The association constants for **2·5** were determined to be 39800 M^{-1} (K_{11}) and 1610 M^{-1} (K_{21}) ($\beta_{21} = 6.41 \times 10^7$ M^{-2}; Table 1). Similar complexation behaviour of β-glucopyranoside **5** could be also observed in water-containing $CDCl_3$ ([receptor]/[water] = 1:6).

During the titrations of **3** with β-glucopyranoside **5** the signal due to the amine NH^A of **3** (see Figure 5, d) was shifted downfield by about 1.5 ppm with strong broadening, whereas the signals for the $CH_3^{E,F}$, $CH_2^{B,C}$ (see Figure 5, c) and pyridine CH protons were moved up- and downfield in ranges of 0.02–0.17 ppm (Table 1). The analysis of the titration data again indicated the formation of complexes with 1:1 and 2:1 receptor–sugar stoichiometries; the binding constants for **3·5** were found to be 18900 M^{-1} (K_{11}) and 2850 M^{-1} (K_{21}) ($\beta_{21} = 5.38 \times 10^7$ M^{-2}).

The interactions between β-glucopyranoside **5** and the receptors **2** and **3** were also investigated on the basis of inverse titrations (see Figure 6). During the titrations of **5** with **2** or **3** the signals due to the OH protons of **5** were shifted downfield with strong broadening and were unobservable after the addition of about 0.1 equiv. of the receptor (see Figure 6, b and d), indicating important contributions of the OH groups of **5** to the complex formation (similar to the titrations of **5** with **1**). The complexation between **5** and the receptor **2** or **3** was further evidenced by significant chemical shift changes of the CH units of **5** (see Figure 6). For example, during the titrations of **5** with **2** the signals due to the 1- and the 2-CH protons of **5** were shifted upfield by 0.23 and 1.75 ppm, respectively. The corresponding titrations of **5** with **3** resulted in upfield shifts of the 1- and 2-CH signals of **5** by 0.19 and 0.95 ppm, respectively. Among the CH signals, the signal due to the 2-CH proton of **5** shows the largest shift, suggesting a particularly important contribution of the CH units to the complex stabilisation (through CH···π interactions with the phenyl ring of the receptor). The participation of the CH units of **5** in

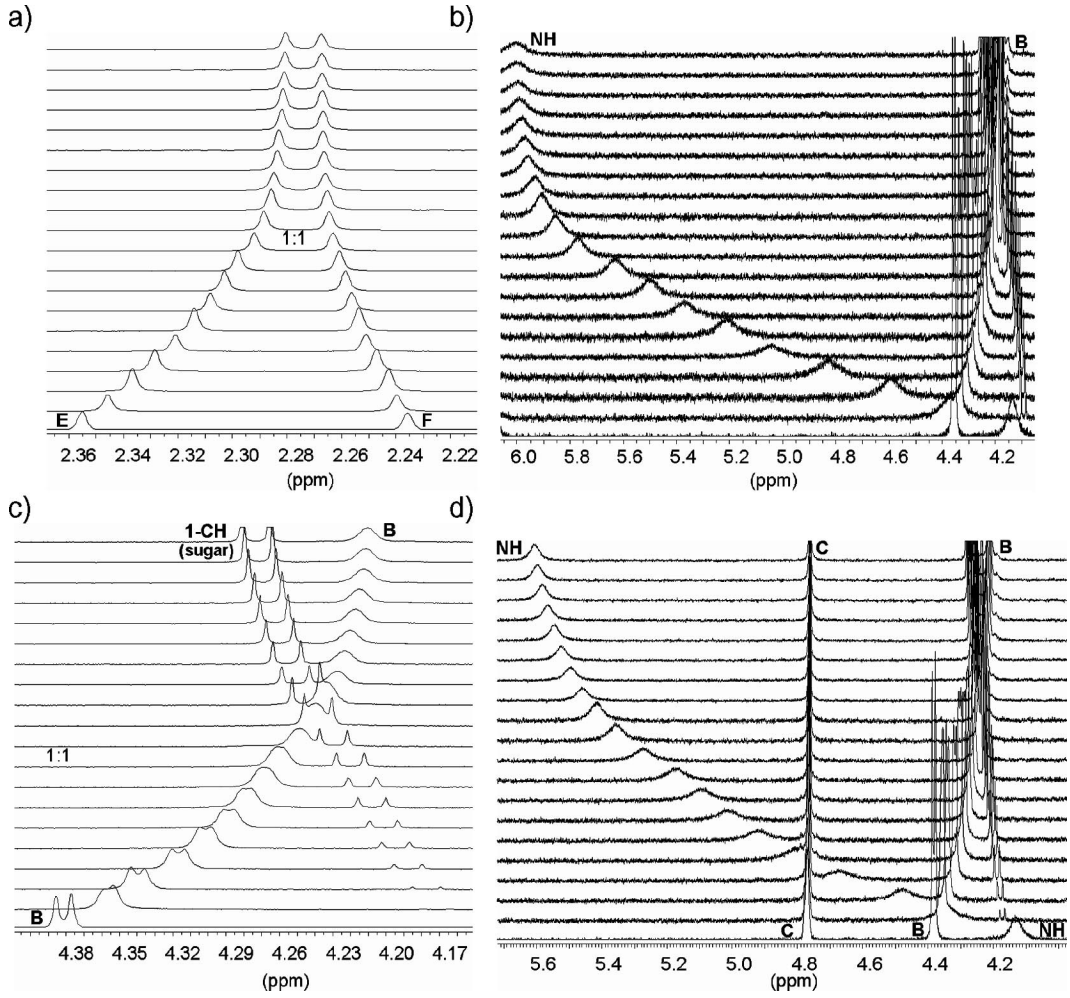


Figure 5. a, b) Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$) of **2** after addition of (from bottom to top) 0.00–3.55 equiv. of β-glucopyranoside **5** ([**2**] = 0.99 mM). Shown are chemical shifts of the $CH_3^{E,F}$ and NH^A resonances of **2** (for labelling, see Figure 2). c, d) Partial ^{1}H NMR spectra of **3** after addition of (from bottom to top) 0.00–3.52 equiv. of **5** ([**3**] = 0.99 mM). Shown are chemical shifts of the $CH_2^{B,C}$ and NH^A resonances of **3**.

CH···π interactions with the central phenyl ring of the receptor was also indicated by molecular modelling (Figure 7). In both cases (**5·2** and **5·3**), the best fits of the titration data were obtained with the "mixed" 1:1 and 1:2 sugar–receptor binding model; thus, the inverse titrations fully confirmed the binding model determined through the titrations of the receptor **2** or **3** with sugar **5**. The association constants obtained on the base of these titrations are again identical within the limits of uncertainty to those determined from titrations in which the roles of receptor and substrate was reversed.

Binding studies with α-glucopyranoside **6** showed the interactions of the receptors **2** and **3** with this monosaccharide to be less favourable than those with β-glucopyranoside **5**. Similarly to the binding studies with sugar **5**, the complexation between receptors **2** and **3** and glucopyranoside **6** was evidenced by several changes in the NMR spectra (see, for example, Figure S5, Supporting Information). However, whereas after the addition of about 2 equiv. of β-glucopyranoside **5** almost no more change in the chemical shifts of the receptor signals was observed, with the monosaccharide **6** chemical shift changes continue to higher [sugar]:[receptor] ratios, indicating lower affinities of **2** and **3** toward **6**. During the titrations of **2** and **3** with α-glucopyranoside **6** the signals due to the amine NH^A of the receptors were moved downfield by about 1.2 ppm (after the addition of about 4.5 equiv. of **6**; see Figure S5, a). Furthermore, the ^{1}H NMR titrations of **2** or **3** with **6** produced chemical shift changes in the $CH_2^{B,C}$ (see, for example, b in Figure S5), $CH_3^{E,F}$ and pyridine CH groups (in ranges of 0.03–0.14 ppm). The curve fitting of the titration data indicated the formation of complexes with 1:1 receptor–sugar stoichiometry. The binding model is different from that determined for the receptors **2**/**3** and β-glucopyranoside **5**. The binding constants were found to be 2180 $M^{-1}$and 1840 M^{-1} for **2·6** and **3·6**, respectively (see Table 1). Thus, the complexes formed between the receptors **2**/**3** and α-glucopyranoside **6** are much less stable than those formed with the β-glucopyranoside **5**. Like receptor **1**, compounds **2** and **3** show high β vs. α binding selectivity in the recognition of glucopyranosides; however, they show significantly lower affinity than the receptor **1** for **5** and **6** (see Table 1).

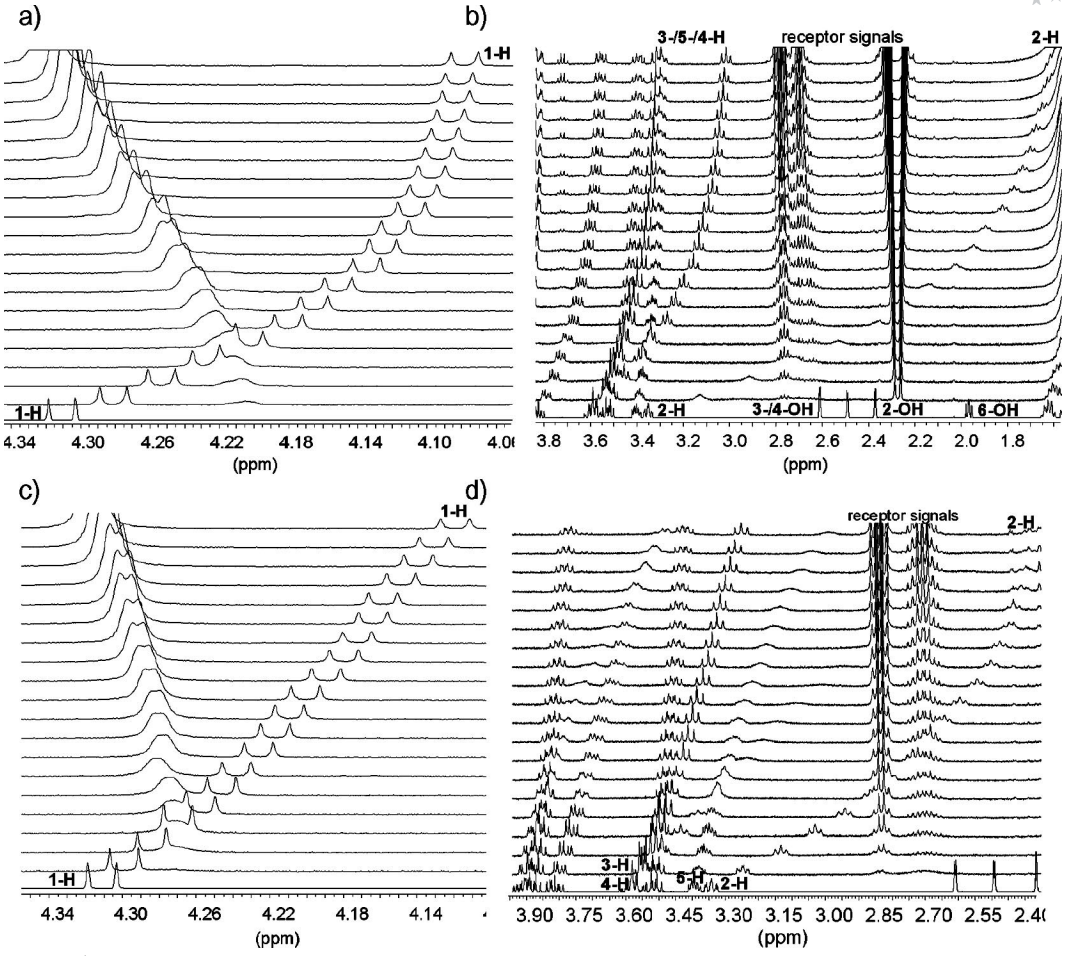


Figure 6. a, b) Partial ^{1}H NMR spectra (500 MHz. $CDCl_3$) of β-glucopyranoside **5** after addition of (from bottom to top) 0.00–4.94 equiv. of **2** ([**5**] = 0.69 mM). c and d) Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$) of β-glucopyranoside **5** after addition of (from bottom to top) 0.00–4.91 equiv. of **3** ([**5**] = 0.70 mM).

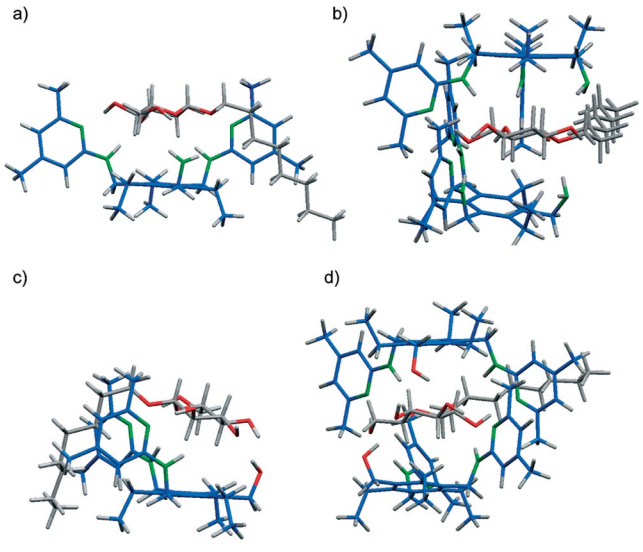


Figure 7. Energy-minimized structure of a) the 1:1 complex formed between receptor **2** and β-glucopyranoside **5**, b) the 2:1 receptor–sugar complex between **2** and **5**, c) the 1:1 complex between receptor **3** and β-glucopyranoside **5**, and d) the 2:1 receptor–sugar complex formed between **3** and **5** (MacroModel V.8.5, OPLS-AA forcefield, MCMM, 50000 steps). Color code: receptor C, blue; receptor N, green; sugar O, red; sugar C,H, grey.

It should be also noted that the receptors **2** and **3** exhibit a level of affinity towards α-glucopyranoside **6** similar to that shown by the previously described three-armed pyridine-based analogue[4a].

Comparative Binding Studies with Compound 4

In contrast with the strong binding of receptors **1**–**3** with β-glucopyranoside **5**, the interactions between compound **4**, lacking the third hydrogen-bonding site, and the sugar **5** are, as might be expected, relatively weak. ^{1}H NMR titrations of **4** with **5** produced several spectral changes (see Figure 8); however, saturation did not occur before the addition of more than 5 equiv. of **5**. The ^{1}H NMR spectra showed changes in the chemical shifts of the NH^A (downfield shift by 0.99 ppm, after the addition of 7.5 equiv. of **5**), CH_2^B (upfield shift by 0.13 ppm), pyridine CH and CH_3 (up- and downfield shifts in ranges of 0.02–0.05 ppm) resonances of **4**. The best fit of the titration data was obtained with the 1:1 binding model; the binding constant for **4**·**5** was found to be 1330 M^{-1}. Thus, the removal of the third hydrogen-bonding site results in a significant fall in the binding constant.

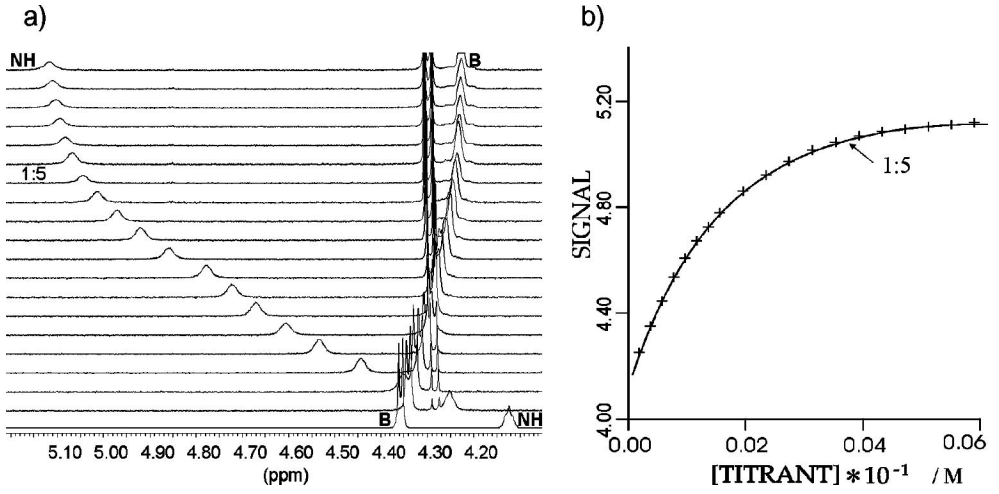


Figure 8. a) Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$) of **4** after addition of (from bottom to top) 0.00–7.42 equiv. of β-glucopyranoside **5** ([**4**] = 0.80 mM). Shown are chemical shifts of the CH_2^B and NH^A resonances of **4** (for labelling, see Figure 4). b) Plot of the observed (×) and calculated (–) chemical shifts of the NH^A signal of **4** as a function of added **5**.

Conclusions

Nature's use of Asn, Gln, Tyr, Ser and Lys to bind carbohydrates, as shown in Figure 1, suggests that combining of primary amide, amino or hydroxy groups (or analogues of the natural recognition groups) should lead to effective carbohydrate receptors. Binding studies with receptors **1**–**3**, containing such hydrogen-bonding groups, as well as 2-aminopyridine units as heterocyclic analogues of the asparagine/glutamine primary amide side chains, have confirmed this hypothesis.

The comparison of the binding properties of the amide-/aminopyridine-based receptor **1** with those of the previously described three-armed pyridine-based analogue {1,3,5-tris[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene}[4a] shows that the incorporation of the two amide units into the acyclic receptor structure significantly affects the binding affinity of the new receptor. Receptor **1** has the tendency to form strong 1:1 and 2:1 receptor–sugar complexes with β-glucopyranoside **5** with an overall binding constant $\beta_{21} \approx 10^8$ M^{-2} (see Table 1). Comparison of the binding isotherms calculated on the base of the stepwise binding constants and the analysis of the mole ratio plots suggested that the 1:1 receptor–monosaccharide complexes predominate in chloroform solution. The interactions of receptor **1** with α-glucopyranoside **6** are less favourable than those with β-glucopyranoside **5**; however, the affinity of **1** toward the α anomer **6** (see Table 1) is much higher than the affinities of the previously described three-armed pyridine-based receptors (see ref.[4a,4c]).

Like compound **1**, the receptors **2** and **3** show high β vs. α binding selectivity in the recognition of glucopyranosides. The binding affinities of the two receptors for the β anomer **5** are high, but significantly lower than that of **1** (see Table 1). Both hydrogen bonding and interactions of the sugar CH groups with the central phenyl rings of the receptors contribute to the stabilisation of the receptor–sugar complexes (as also indicated by molecular modelling calculations; see Figure 7).

The removal of the third hydrogen-bonding site results in a significant fall in the binding constant, as shown by comparative binding studies with compound **4**, which incorporates only two aminopyridine groups as hydrogen-bonding units (see Table 1). The incorporation of the primary amide, amino or hydroxy group into the receptor structure thus significantly affects the binding affinities of the receptors **1**–**3**.

The selective and effective recognition of neutral sugars by artificial receptors still represents a significant challenge, so synthetic receptors using noncovalent interactions provide valuable model systems to study the basic molecular features of carbohydrate recognition. Both our previous studies and the present investigations show that the acyclic receptors represent particularly interesting objects for such studies. The acyclic scaffold provides simplicity in the synthetic plan for many modifications of the receptor structure, supplying a base for systematic studies toward recognition motifs for carbohydrates.

Experimental Section

General: Analytical TLC was carried out on silica gel 60 F_{254} plates with ethyl acetate/toluene (3:1, v/v) or chloroform/methanol (7:1, v/v) as the mobile phase. Melting points are uncorrected.

1-(Bromomethyl)-3,5-bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (7): A CH_3CN (10 mL) solution of 2-amino-4,6-dimethylpyridine (3.16 g, 25.69 mmol) was added to a mixture of 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene (6.00 g, 13.60 mmol) and K_2CO_3 (3.76 g, 27.20 mmol) in CH_3CN/THF (1:1, v/v; 40 mL).The mixture was stirred at room temperature for 48 h. After filtration and removal of solvents, the crude product was purified by column chromatography (ethyl acetate/toluene, 1:3, v/v). Yield 2.13 g (30%); m.p. 78–79 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.22 (t, J = 7.5 Hz, 3 H), 1.29 (t, J = 7.5 Hz, 6 H), 2.24 (s, 6

H), 2.36 (s, 6 H), 2.73 (q, J = 7.5 Hz, 2 H), 2.85 (q, J = 7.5 Hz, 4 H), 4.23 (br s, 2 H), 4.37 (d, J = 4.2 Hz, 4 H), 4.62 (s, 2 H), 6.10 (s, 2 H), 6.35 (s, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.4, 16.7, 21.1, 22.8, 23.0, 24.1, 29.6, 40.5, 103.6, 113.9, 131.9, 133.4, 143.8, 144.9, 148.9, 156.5, 158.0 ppm. HR-MS calcd. for $C_{29}H_{39}BrN_4$: 522.2353; found 522.2360. R_f = 0.12 (ethyl acetate/toluene, 1:3).

1-(Aminomethyl)-3,5-bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (2): Aqueous ammonia solution (25%, 25 mL) was added to a solution of **7** (776 mg, 1.48 mmol) in THF/MeOH (1:1, v/v, 20 mL). This mixture was stirred at room temperature for 12 h. After evaporation of solvents, water (20 mL) was added, and the solution was extracted with $CHCl_3$ (3 × 20 mL). The combined organic phases were washed with brine (20 mL) and dried with $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography ($CHCl_3$/MeOH, 7:1, v/v). Yield 0.49 g (72%); m.p. 82–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.22 (t, J = 7.3 Hz, 3 H), 1.23 (t, J = 7.4 Hz, 6 H), 2.23 (s, 6 H), 2.36 (s, 6 H), 2.72 (q, J = 7.3 Hz, 2 H), 2.80 (q, J = 7.4 Hz, 4 H), 3.92 (s, 2 H), 4.36 (s, 6 H), 6.10 (s, 2 H), 6.34 (s, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 16.76, 16.81, 21.15, 22.48, 22.76, 23.97, 39.47, 40.63, 103.61, 113.82, 133.15, 135.02, 142.07, 142.60, 155.22, 158.27 ppm. HR-MS calcd. for $C_{29}H_{41}N_5$: 459.3356; found 459.3352. R_f = 0.17 ($CHCl_3$/CH_3OH, 7:1).

3,5-Bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethyl-1-(hydroxymethyl)benzene (3): Aqueous NaOH (366.7 mg, 9.15 mmol in 10 mL of H_2O) was added to a solution of **7** (1.2 g, 2.29 mmol) in THF (20 mL). The solution was heated at reflux for five hours. After evaporation of the THF, the remaining water phase was extracted with chloroform (3 × 20 mL). The combined organic phases were dried with $MgSO_4$. After evaporation of the solvent, the obtained powder was purified by column chromatography ($CHCl_3$/CH_3OH, 7:1). Yield 0.80 g (75%); m.p. 75–76 °C. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.13 (t, J = 7.5 Hz, 3 H), 1.16 (t, J = 7.4 Hz, 6 H), 2.08 (s, 6 H), 2.26 (s, 6 H) 2.69 (q, J = 7.5 Hz, 2 H), 2.79 (q, J = 7.4 Hz, 4 H), 4.38 (d, J = 4.1 Hz, 4 H), 4.56 (d, J = 4.2 Hz, 2 H), 4.78 (t, J = 4.2 Hz, 1 H), 5.93 (t, J = 4.1 Hz, 2 H), 6.19 (s, 2 H), 6.21 (s, 2 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 16.52, 16.61, 20.48, 22.13, 22.50, 24.08, 38.86, 56.97, 105.30, 112.01, 132.86, 135.21, 142.59, 142.83, 146.65, 155.19, 158.29 ppm. HR-MS calcd. for $C_{29}H_{40}N_4O$: 460.3196; found 460.3187. R_f = 0.17 ($CHCl_3$/CH_3OH, 7:1).

1-{[Bis(ethoxycarbonylmethyl)]aminomethyl}-3,5-bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (8): K_2CO_3 (212 mg, 1.52 mmol) was added to a solution of compound **7** (800 mg, 1.52 mmol) in THF (15 mL) and CH_3CN (15 mL). Diethyl iminodiacetate (0.29 g, 0.34 mL, 1.52 mmol) was added to this suspension. The reaction mixture was stirred at room temperature for 24 h and was then filtered. After removal of the solvents the crude product was purified by column chromatography ($CHCl_3$/CH_3OH, 7:1). Yield 0.58 g (60%); m.p. 52–53 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18 (t, J = 7.5 Hz, 6 H), 1.23 (t, J = 7.6 Hz, 3 H), 1.26 (t, J = 7.1 Hz, 6 H), 2.23 (s, 6 H), 2.34 (s, 6 H), 2.72 (q, J = 7.6 Hz, 2 H), 2.89 (q, J = 7.5 Hz, 4 H), 3.53 (s, 4 H), 4.01 (s, 2 H), 4.13 (q, J = 7.1 Hz, 4 H), 4.24 (br s, 2 H), 4.35 (d, J = 4.2 Hz, 4 H), 6.31 (s, 2 H), 6.33 (s, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.14, 16.41, 16.68, 21.04, 22.48, 22.80, 24.07, 40.56, 50.98, 53.13, 60.31, 103.20, 113.72, 131.69, 132.77, 143.45, 144.78, 148.70, 156.60, 158.22, 171.36 ppm. HR-MS calcd. for $C_{37}H_{53}N_5O_4$: 631.4092; found 631.4098. R_f = 0.22 ($CHCl_3$/CH_3OH 7:1).

1-{[Bis(carbamoylmethyl)]aminomethyl}-3,5-bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (1): Ammonia solution (7 N in methanol, 10 mL) was added to a solution of compound **8** (400 mg, 0.63 mmol) in methanol (10 mL). The reaction mixture was stirred for 48 h. During this time a solid precipitated. The solid was filtered off and was purified by column chromatography ($CHCl_3$/CH_3OH, 7:1). Yield 0.14 g (39%); m.p. 106–107 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.10 (t, J = 7.3 Hz, 6 H),1.16 (t, J = 7.4 Hz, 3 H), 2.09 (s, 6 H), 2.26 (s, 6 H), 2.66 (q, J = 7.4 Hz, 2 H), 2.85 (q, J = 7.3 Hz, 4 H), 3.33 (s, 4 H), 3.74 (s, 2 H), 4.38 (d, J = 4.0 Hz, 4 H), 6.03 (br s, 2 H), 6.19 (s, 2 H), 6.22 (s, 2 H), 7.09 (s, 2 H), 7.46 (s, 2 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 16.37, 16.43, 20.53, 21.97, 22.56, 23.97, 38.87, 51.36, 56.69, 105.37, 112.18, 130.92, 132.95, 143.11, 143.90, 146.90, 154.99, 158.14, 172.69 ppm. HR-MS calcd. for $C_{33}H_{47}N_7O_2$: 573.3785; found 573.3777. R_f = 0.16 ($CHCl_3$/CH_3OH, 7:1).

1,3-Bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (4): K_2CO_3 (316 mg, 2.30 mmol) was added to a solution of 1,3-bis(bromomethyl)-2,4,6-triethylbenzene[10a] (400 mg, 1.15 mmol) in THF (20 mL) and CH_3CN (10 mL). 2-Amino-4,6-dimethylpyridine (281 mg, 2.30 mmol) dissolved in CH_3CN (10 mL) was added to this suspension. The reaction mixture was stirred at room temperature for 48 h and was then filtered. After removal of the solvents the crude product was purified by column chromatography (toluene/ethyl acetate, 3:1, v/v). Yield 0.38 g (76%); m.p. 157–158 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.21 (t, J = 7.5 Hz, 3 H), 1.23 (t, J = 7.5 Hz, 6 H), 2.22 (s, 6 H), 2.34 (s, 6 H), 2.70 (q, J = 7.5 Hz, 4 H), 2.76 (q, J = 7.5 Hz, 2 H), 4.15 (t, J = 4.0 Hz, 2 H), 4.36 (d, J = 4.0 Hz, 4 H), 6.08 (s, 2 H), 6.33 (s, 2 H), 6.99 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 15.93, 16.92, 21.06, 22.57, 24.17, 26.11, 40.27, 103.39, 113.78, 127.36, 132.00, 143.31, 143.57, 148.67, 156.70, 158.33 ppm. HR-MS calcd. for $C_{28}H_{38}N_4$: 430.3091; found 430.3083. R_f = 0.05 ($CHCl_3$/CH_3OH, 7:1).

Supporting Information (see also the footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of compounds **1**–**4**; representative mole ratio plots; further examples of ^{1}H NMR titrations.

Acknowledgments

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