ROYAL SOCIETY OF CHEMISTRY

PAPER

Carbohydrate receptors combining both a macrocyclic building block and flexible side arms as recognition units: binding properties of compounds with CH₂OH groups as side arms†

Felix Amrhein, Jan Lippe and Monika Mazik*

New representatives of compounds combining both a macrocyclic building block and two flexible side arms as recognition units were prepared and their binding properties toward selected carbohydrates were evaluated. The aim of this study was to examine the effects of the replacement of the heterocycle-bearing side arms by smaller units, such as hydroxy groups, on the binding capability. The design of this type of receptor was inspired by the participation of the side chain hydroxy group of serine and threonine in the biorecognition of carbohydrates. Such structural modifications enable the recognition of structure–activity relationships, which are of high importance in the development of carbohydrate receptors with predictable binding strength and selectivity.

Introduction

Inspired by the binding motifs observed in the crystal structure of the 2 : 1 receptor–glucopyranoside complex formed by an acyclic trimethylbenzene-based receptor bearing three heterocyclic recognition groups,[1] we have designed a new type of effective artificial carbohydrate receptor consisting of a macrocyclic building block and two flexible side arms (see Fig. 1).[2,3] Through the use of different types of bridges (units Y in Fig. 1) and side arms (units X) as building blocks, we succeeded in developing various receptors with interesting binding properties (see structures **1–11** in Fig. 2). The desired ability to form effective and selective 1 : 1 complexes with selected carbohydrates,[2,3] instead of 2 : 1 receptor–sugar complexes as in the case of the acyclic receptor,[1] could be achieved with the new receptor architecture, as shown exemplarily for compound **4** and octyl-β-ᴅ-glucoside in Fig. 3. Compounds bearing aminopyridine or -pyrimidine groups as flexible side arms and pyrrole-/benzene-based bridges (**1–5**) were shown to

*Institut für Organische Chemie, Technische Universität Bergakademie Freiberg,
Leipziger Strasse 29, 09599 Freiberg, Germany.
E-mail: monika.mazik@chemie.tu-freiberg.de; Fax: +03731393170;
Tel: +03731392389*

†Electronic supplementary information (ESI) available: Descriptions of the binding studies (extraction experiments, ^{1}H NMR spectroscopic titrations and microcalorimetric titrations). Further examples of molecular modelling calculations (Fig. S1–S3). Schematic representations of some short contacts between β-glucoside **24** and receptor **13** or **14** indicated by ROESY spectra (Fig. S4). Copies of the ^{1}H and ^{13}C NMR spectra of the new compounds **12–14**, **17** and **18** (Fig. S5–S14). See DOI: 10.1039/c6ob01682k

be more effective receptors than their analogues consisting of pyridine- or hydroxybenzene-based bridges (compounds **8–11**, see Fig. 4). The considerable lower affinity of compounds **8–11** is mainly a consequence of the participation of their bridge units in intramolecular hydrogen bonds (particularly drastic reduction of the binding affinity was observed for compounds consisting of hydroxybenzene units). Furthermore, the involvement of the flexible side arms in intramolecular interactions, as in the case of compounds incorporating 8-hydroxyquinoline-based side arms (compounds **6** and **7**),[3] also results in a reduction of the binding affinity (in comparison to their analogues bearing aminopyridine and aminopyrimidine groups).
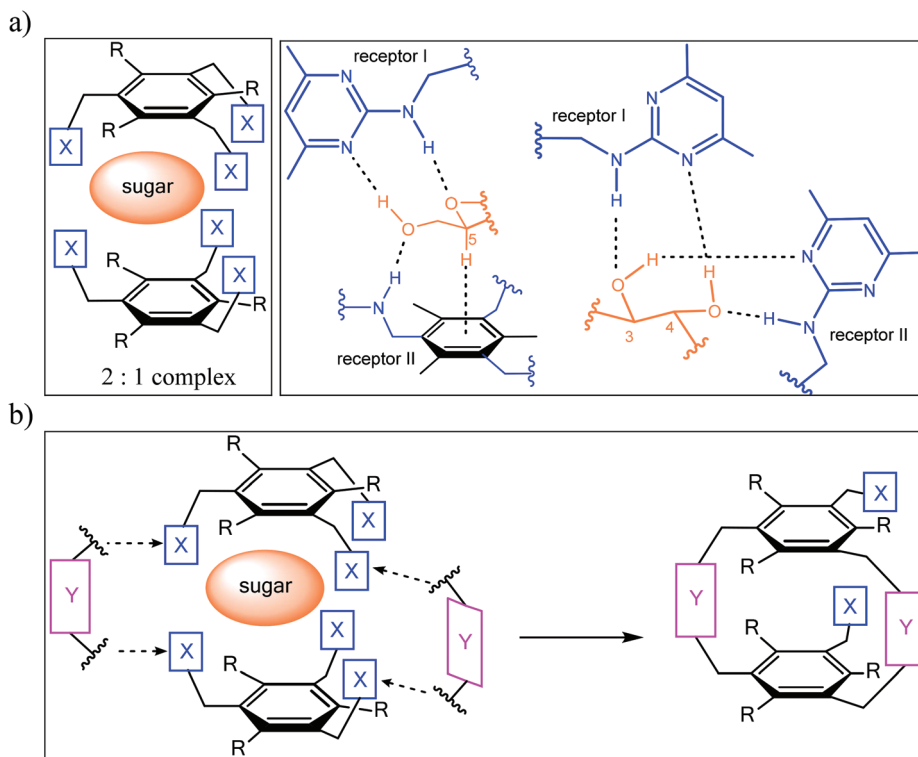
Our previous studies clearly showed the enormous potential of the designed receptor architecture for versatile structure variations through the combination of different types of bridges and side arms. In addition, the replacement of the two triethylbenzene units by other aromatic cores, having the ability to participate in CH···π interactions[4–6] with the sugar CH units, provides further possibilities for structural modifications. Such versatile possibilities for structural changes enable the identification of structure–activity relationships, which are of particular importance for the design of artificial receptors with predictable binding strength and selectivity. It should be stressed at this point that the development of carbohydrate receptors with exact predictable binding properties still represents a great challenge.[7]

We were interested to see how the replacement of the heterocycle-bearing side arms by smaller units will affect the binding properties of the new compounds. The participation of the side chain hydroxy group of serine and threonine in
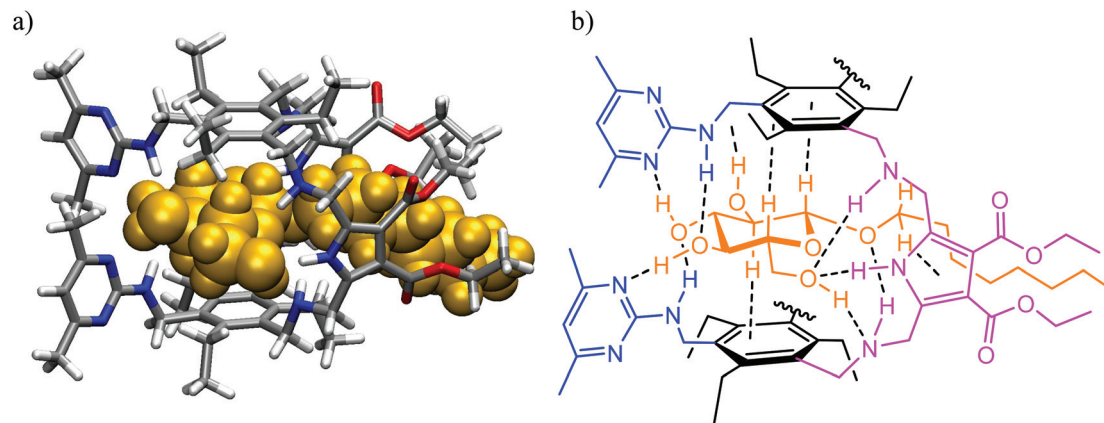
This journal is © The Royal Society of Chemistry 2016

Fig. 1 (a) Examples of hydrogen bonding and CH−π interactions in the crystalline 2 : 1 complex between an acyclic trimethylbenzene-based receptor (X = 2-amino-4,6-dimethylpyrimidine, R = Me) and octyl β-D-glucoside. (b) Schematic illustration of the design principle[2,3] of carbohydrate receptors combining both a macrocyclic building block (units Y) and flexible side arms (units X).
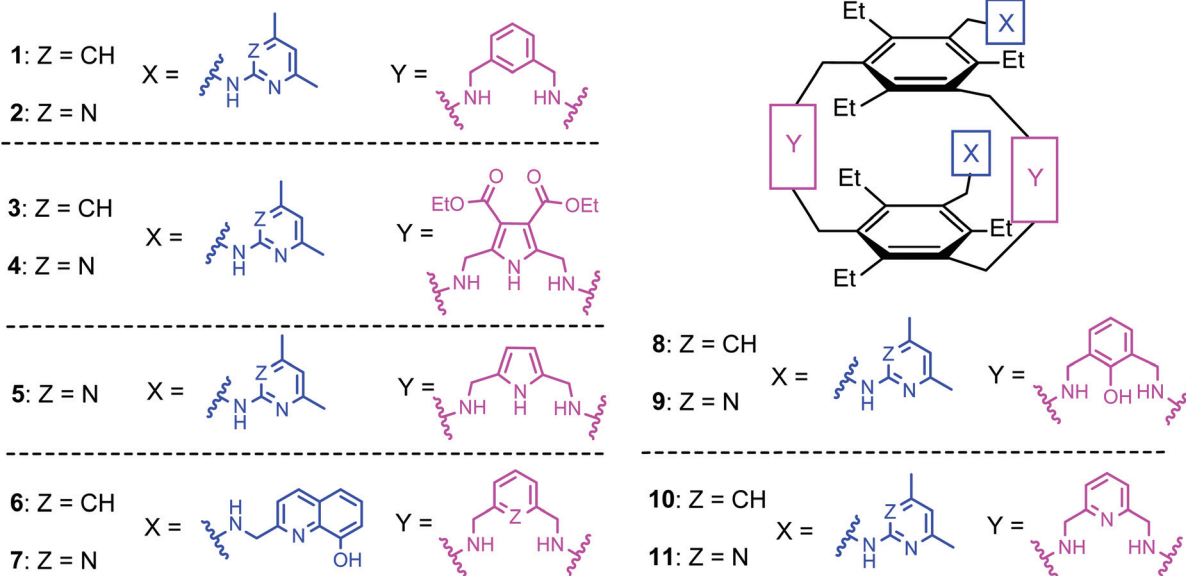

Fig. 2 Structures of the previously described macrocyclic compounds **1–11** containing two flexible side arms as recognition units.

the biorecognition of carbohydrates,[8–10] as shown exemplarily in Fig. 5, has inspired us to design receptors bearing hydroxy groups[11–14] as recognition sites. The aim of the present study was the synthesis and the evaluation of the binding

properties of compounds **12–14** incorporating hydroxymethyl groups as flexible side arms (Fig. 6). Based on the results of our previous studies, we have selected benzene- and pyrrole-based subunits as bridges, since these building blocks led to

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10648–10659 | **10649**

Fig. 3 (a) Energy-minimized structure of the 1 : 1 complex formed between the receptor **4** and octyl β-D-glucoside (MacroModel V.8.5, OPLS 2001 force field, MCMM, 50 000 steps). Color code: receptor N, blue; receptor O, red; receptor C, gray; the sugar molecule is highlighted in orange. (b) Schematic representation of the binding motifs in the complex (shown are interactions indicated by molecular modelling and confirmed by NMR spectroscopy).[3]
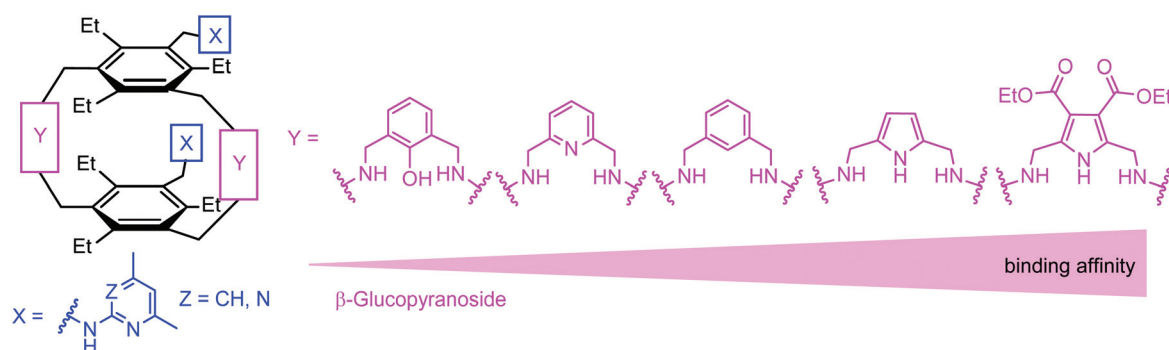
Fig. 4 Schematic illustration of the influence of the bridge type (units Y) on the binding strength toward β-glucopyranoside in the case of macro-cyclic compounds bearing two aminopyridine or -pyrimidine groups as flexible side-arms (units X).
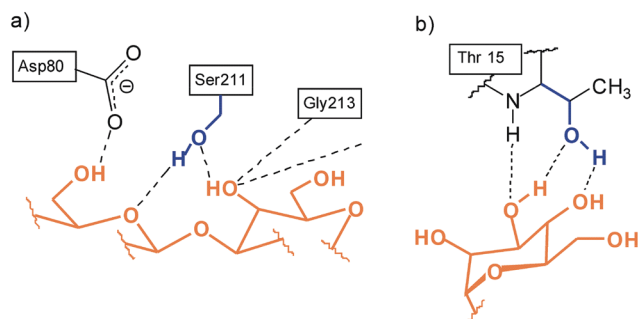
Fig. 5 Examples for the participation of the side chain hydroxy group of serine and threonine in biorecognition of carbohydrates: (a) Galβ3GlcNAc in the combining site of peanut agglutinin;[8,10a] (b) neutral hydrogen bonds in the complex between Manα6(Manα3)Man and concanavaline A.[9,10a]

the construction of particularly powerful carbohydrate receptors.

Compared to the earlier compounds **1–5**, consisting of two aminopyridine- or aminopyrimidine-based side arms, which

possess the ability to form more extensive interactions with the sugar substrate than the hydroxy groups of **12–14** (see Fig. 7a), a reduction in the binding capability of the new compounds was anticipated. In view of the potential of the pyrrole-based bridges[15] to create more favourable intermolecular interactions than the benzene-bearing units (see Fig. 7b), compounds **13** and **14** were expected to be more powerful receptors than their analogue **12**. Studies with compounds **12–14** confirmed these assessments and revealed again that the combination of a macrocyclic building block and two flexible side arms represents a particular valuable strategy for the construction of carbohydrate receptors with tunable binding properties.

Results and discussion

Synthesis

Compounds **12–14** were prepared on the basis of 3,5-bis(aminomethyl)-1-hydroxymethyl-2,4,6-triethylbenzene (**18**), the preparation of which started from 1,3,5-tris(bromomethyl)-
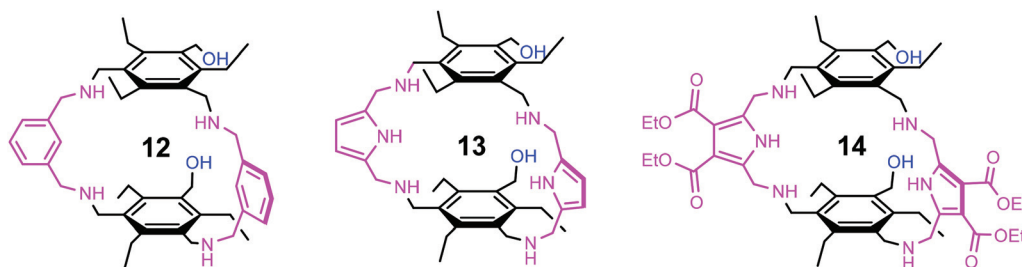
10650 | *Org. Biomol. Chem.*, 2016, **14**, 10648–10659

This journal is © The Royal Society of Chemistry 2016

Fig. 6 Structures of the macrocyclic compounds **12–14** bearing two hydroxymethyl-groups as flexible side arms.
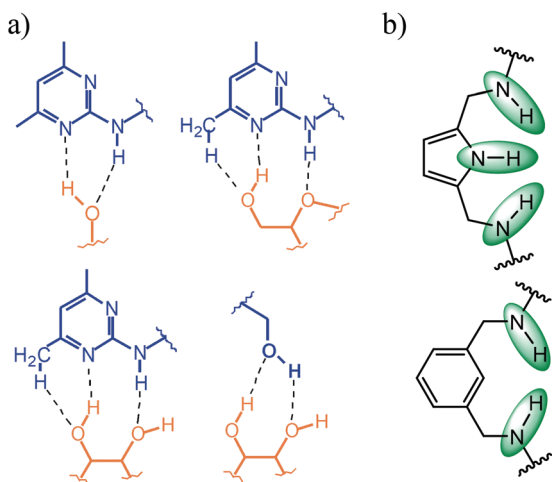

Fig. 7 (a) Examples of noncovalent interactions, which can be formed by 2-amino-4,6-dimethylpyridine and the hydroxy group with carbohydrates; (b) hydrogen bonding sites (see green marking) of pyrrole- and benzene-based bridges (units Y in Fig. 1).

2,4,6-triethylbenzene (**15**), as shown in Scheme 1. The reaction of **15** with potassium phthalimide (**16**) in water-containing dimethyl sulfoxide provided 1-hydroxymethyl-3,5-bis(phthalimidomethyl)-2,4,6-triethylbenzene (**17**) and 1,3,5-tris(phthalimidomethyl)-2,4,6-triethylbenzene, which could be separated out from each other by means of flash chromatography. The best yield of compound **17** (43%) could be achieved by using three equivalents of potassium phthalimide and 7%–10% water in dimethyl sulfoxide. The decrease in water content to 2–3% caused a reduction in the yield of **17**. The use of only two equivalents of potassium phthalimide significantly decreased the yield of **17** to around 10%.

Compound **17** was converted into 3,5-bis(aminomethyl)-1-hydroxymethyl-2,4,6-triethylbenzene (**18**) by treatment with hydrazine hydrate. The condensation of **18** with the corresponding carbaldehyde, such as benzene-1,3-dicarbaldehyde (**19**), 1H-pyrrole-2,5-dicarbaldehyde (**20**) or diethyl 2,5-diformyl-1H-pyrrole-3,4-dicarboxylate (**21**), provided the corresponding imines (compounds **12a**, **13a** and **14a**), which were further reduced with sodium borohydride to give the target

compounds **12–14**. The imines **12a** and **13a** were isolated *via* filtration and after their reduction the products **12** and **13** could be isolated in 86% and 83% yield, respectively. Due to the good solubility of the imine **14a**, this imine was directly reduced without isolation. Product **14** was purified by column chromatography on silica gel using a chloroform/methanol mixture as the eluant and could be isolated in 41% yield. 1H-Pyrrole-2,5-dicarbaldehyde (**20**) and diethyl 2,5-diformyl-1H-pyrrole-3,4-dicarboxylate (**21**), used for the synthesis of compounds **13** and **14**, respectively, were prepared according to known procedures.[16,17]
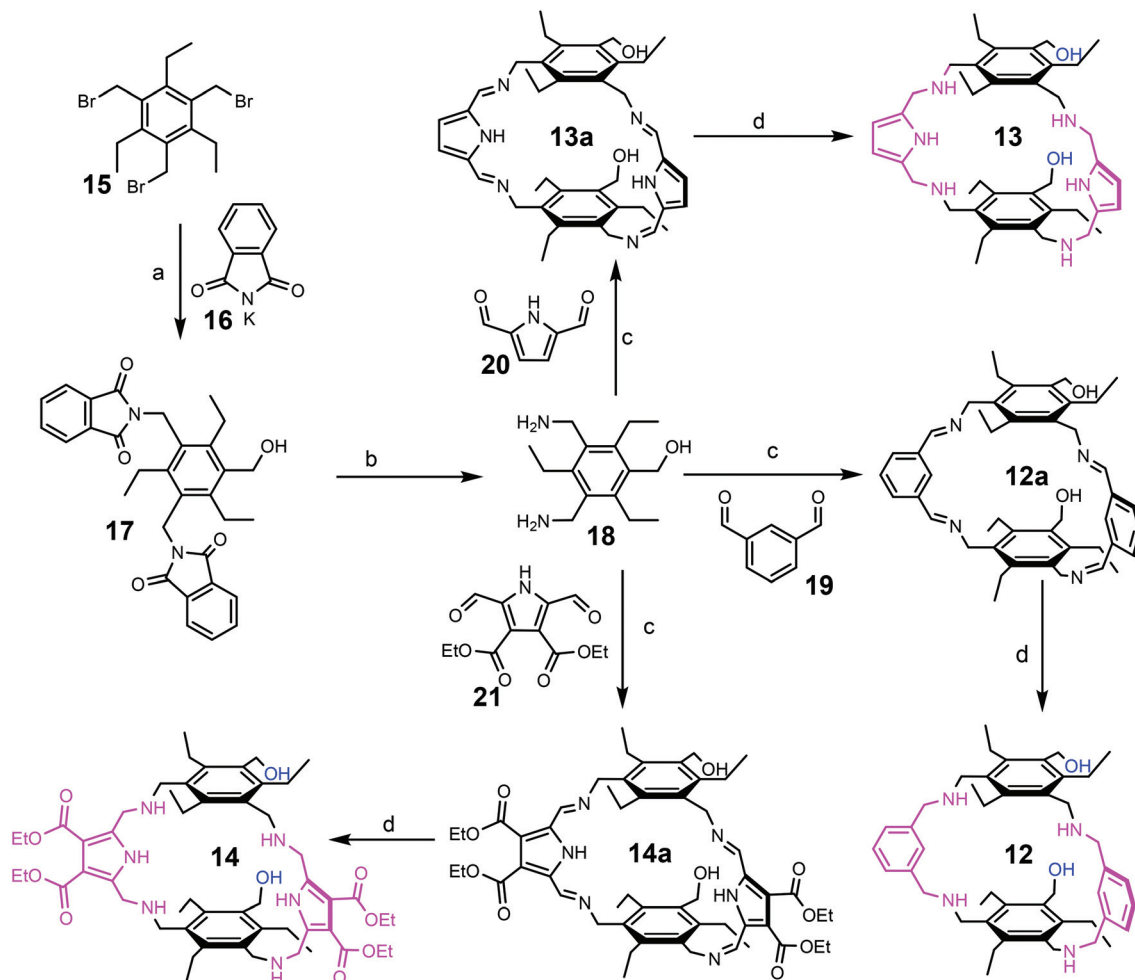
Binding studies

To compare the binding properties of the new compounds with those of the previously studied receptors, methyl β-ᴅ-glucoside (**22**) and methyl α-ᴅ-glucoside (**23**) were employed for binding studies in two-phase systems (extraction of the corresponding sugar from water into the organic phase), whereas octyl β-ᴅ-glucoside (**24**) was selected as a substrate for the binding studies in homogeneous media (1H NMR spectroscopic titrations and microcalorimetric titrations). Furthermore, ROESY studies were carried out and provided structural information for the receptor–sugar complexes.

Extraction experiments: phase transfer of sugars from aqueous into organic solvents

Similarly to the case of the previously studied compounds **1–5**, the extraction of methyl β-ᴅ-glucoside (**22**) and methyl α-ᴅ-glucoside (**23**) from aqueous into chloroform solution revealed a binding preference for β-glucoside **22**, *i.e.* for a substrate with all-equatorial substitution pattern (similar to the receptors reported by Davis *et al.*[18]). Compared to the pyrimidine-bearing analogue **5**, compound **13** showed a decreased affinity for β-glucoside **22** and was able to extract only 0.44 equiv. of the sugar (*versus* 0.74 equiv. for **5**). It should however be noted that similar extractability was observed in the case of compounds **1** and **2** (0.40 and 0.50 equiv. of **22** for **1** and **2**, respectively), consisting of pyridine- or pyrimidine-bearing side arms and benzene-based bridges. Thus, depending on the used combination of the side arms and bridges (X/Y combination), the extractabilities of β-glucoside by compounds **1**, **2**, **4**, **5** and **13** decrease in the following sequence (see Table 1): aminopyri-

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10648–10659 | **10651**

Scheme 1 Reaction conditions: (a) potassium phthalimide (**16**), DMSO, 7–10% H_2O; 43% of **17**, (b) N_2H_4, EtOH/toluene; 91% of **18**, (c) EtOH or ETOH/CH_2Cl_2, AcOH (catalytic amount), (d) $NaBH_4$, MeOH; then H_2O; 86% of **12**, 83% of **13** and 41% of **14** (overall yields for step c and d).

Table 1 Extractabilities of methyl glycosides from aqueous solution (1 M) into $CDCl_3$ by compounds **1**, **2**, **4**, **5** and **13** (1 mM $CDCl_3$ solutions)[a]

Receptor	Combination of the building blocks X/Y[c]	β-Glucoside **22**	α-Glucoside **23**	β-Galactoside
4[b]	Aminopyrimidine/pyrrole(diester)	0.81	0.45	0.50
5[b]	Aminopyrimidine/pyrrole	0.74	0.38	0.58
2[b]	Aminopyrimidine/benzene	0.50	n.d.[d]	0.40
1[b]	Aminopyridine/benzene	0.40	0.15	0.36
13	Hydroxy group/pyrrole	0.44	0.17	n.d.[d,e]

[a] Values in mole equivalents with respect to the receptor; the ^{1}H NMR signals of the corresponding sugar were integrated with respect to the receptor's signals to provide the sugar–receptor ratio (control experiments were performed in the absence of the receptor). [b] Results from ref. 2 and 3. [c] X = side arm unit, Y = bridge unit (pyrrole- or benzene-based, see Fig. 1). [d] Not determined. [e] Overlapping of sugar and receptor signals prevented the estimation of their ratio.

midine/pyrrole > aminopyrimidine/benzene > hydroxy group/ pyrrole ≥ aminopyridine/benzene.

^{1}H NMR spectroscopic titration and microcalorimetric titrations

The ^{1}H NMR spectroscopic titrations with octyl β-D-glucoside (**24**) were carried out by adding increasing amounts of the sugar to a $CDCl_3$ solution of the corresponding receptor **12–14**

(according to the protocol described in the ESI†). The complexation between the binding partners was evidenced by several changes in the NMR spectra (for examples, see Fig. 8). In the case of compounds **13** and **14**, bearing pyrrole-based bridges, the sequential additions of β-glucoside **24** caused successive replacement of the receptor signals by a new set of signals, as shown in Fig. 8a and b. Such spectral changes were also observed for the powerful receptors **2–5** [2,3] and are consist-
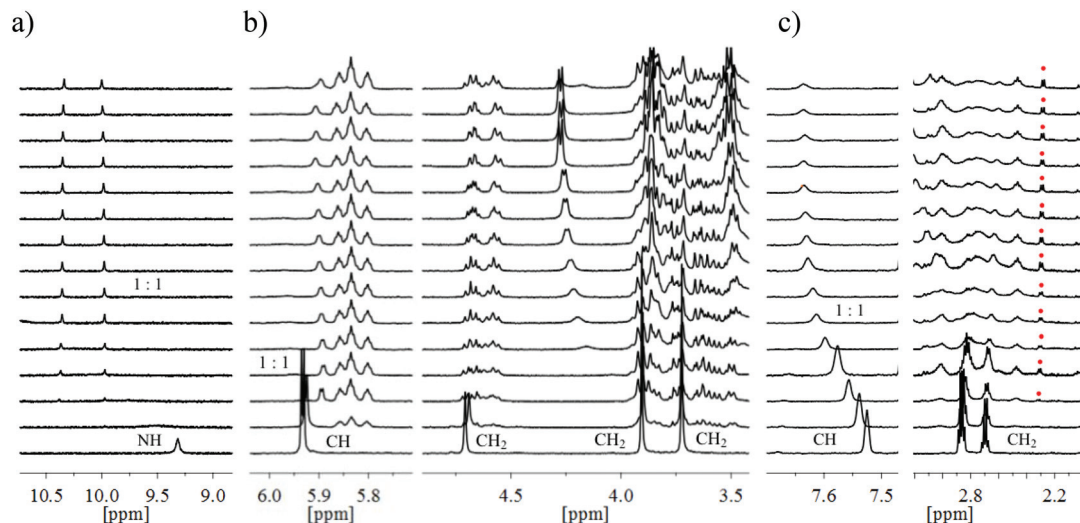
Fig. 8 Partial ^1H NMR (CDCl$_3$, 500 MHz) spectra of receptors **14** (a), **13** (b) and **12** (c) after the addition of 0.00–3.50 equiv. of β-glucoside **24**; [receptor] = 1.0 or 1.1 mM. (a, b) Complex formation with slow equilibration on the NMR time-scale, (c) simultaneous presence of fast- and slow-exchanging (red mark) complexes. Shown are the chemical shifts of the following signals of **12–14**: pyrrole NH of **14**, pyrrole CH and CH$_2$ (side arms and bridge units) of **13** as well as benzene CH (bridge) and CH$_2$ (ethyl groups) of **12**.

ent with complex formation, in which exchange between bound and unbound forms is slow on the NMR time-scale. The estimation of the binding constants directly from the relative ratios of the free and bound receptor provided indication of strong binding ($K_{11} > 100\,000$ M^{-1}); however, significant errors did not allow the accurate determination of the binding constants on the basis of the ^1H NMR data. For the previously tested compounds **2–5** the binding constants were determined on the basis of fluorescence titrations and found to be in the range of 210 000–390 000 M^{-1} in CDCl$_3$,[2,3] this technique could not be used for compounds **13** and **14**.

The spectra of compound **12**, bearing benzene-based bridges, upon addition of β-glucoside **24** showed both significant movements of the receptor signals and the appearance of a new set of signals, as exemplarily shown in Fig. 8c. These results suggest complex formation with both fast and slow equilibration on the NMR time-scale (see also below, Fig. 11). Such a simultaneous presence of fast- and slow-exchanging complexes prevented quantitative calculation of the binding constants. It should, however, be noted that the analysis of the complexation-induced shifts for the predominant fast-exchanging complex provided clear indication for relatively weak interactions between **12** and **24** (K_{11} of about 8000 M^{-1}).

The binding properties of compounds **12** and **13** were further analysed by isothermal titration calorimetry (ITC). The microcalorimetric titrations were carried out in CDCl$_3$ and water-containing CDCl$_3$ at 25 °C by adding increasing amounts of the sugar **24** to a solution of the corresponding receptor (according to the protocol described in the ESI†). Experimental parameters, such as concentration, temperature and calorimeter type (see the ESI†), were strictly maintained to be constant to allow the accurate comparison of the calori-

metric data. The binding constants were determined from three independent microcalorimetric titrations and in all cases the best fit of the titration data was obtained with the 1 : 1 receptor–sugar binding model.

The experiments showed that **12** and **13** form enthalpy driven complexes with sugar **24** not only in dry CDCl$_3$ but also in water-containing CDCl$_3$ (0.035 and 0.07 mol L^{-1} H$_2$O in CDCl$_3$). The enthalpic driving force of all the investigated binding processes is partially compensated by a negative ΔS. As already indicated by the results of the NMR titrations, compound **13** was found to be a much more effective receptor for **24** than compound **12**. The binding constants in dry CDCl$_3$ were determined to be approximately 200 000 M^{-1} and 7900 M^{-1} for **13·24** and **12·24**, respectively (see Table 2 and Fig. 9). When the two bridge units are varied from benzene- (compound **12**) to pyrrole-based units (compound **13**), the increased gain in binding free enthalpy $-\Delta G$ (−22.2 kJ mol^{-1} for **12·24** compared with −30.2 kJ mol^{-1} for **13·24**) originates from a more favourable enthalpic term ($\Delta H \approx -78$ kJ mol^{-1} for **12·24** compared with $\Delta H \approx -87$ kJ mol^{-1} for **13·24**).

In the case of compound **13** the addition of water led to a significant decrease in binding strength and a less favourable complexation enthalpy (see Table 2), but a more favourable entropy term. The partial enthalpy–entropy compensation is also responsible for the relatively small variation in the free energy observed for the binding processes in the presence of 0.035 and 0.07 mol L^{-1} H$_2$O in CDCl$_3$.

In contrast to **13**, compound **12** was found to exhibit a slightly higher binding constant in water-containing CDCl$_3$ compared to that in dry solvent. The drop in the enthalpic driving force for binding is compensated by an increase in entropy in the water-containing solvent (resulting mostly from the desolvation process).

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10648–10659 | **10653**

Table 2 Results of microcalorimetric titrations of compounds **12** and **13** with octyl β-D-glucoside (**24**) in dry $CDCl_3$ and $H_2O/CDCl_3$ (0.035 and 0.07 mol L^{-1} H_2O) at 25 °C[a,b]

| Compound (solvent)	lg K_{11} (K_{11} [M^{-1}])	ΔG [kJ mol^{-1}]	ΔH [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]	ΔS [J mol^{-1} K^{-1}]
13 (dry $CDCl_3$)	5.29 ± 0.02 (195 000 ± 9000)	−30.2 ± 0.2	−87.3 ± 0.9	−57.1	−192
13 (0.035 mol L^{-1} H_2O)	4.94 ± 0.01 (87 100 ± 2000)	−28.2 ± 0.1	−56.8 ± 1.2	−28.6	−96
13 (0.07 mol L^{-1} H_2O)	4.75 ± 0.04 (56 200 ± 5000)	−27.1 ± 0.5	−36.8 ± 0.7	−9.7	−33
12 (dry $CDCl_3$)	3.90 ± 0.02 (7900 ± 400)	−22.2 ± 0.2	−78.6 ± 0.3	−56.2	−190
12 (0.07 mol L^{-1} H_2O)	4.06 ± 0.02 (11 500 ± 500)	−23.1 ± 0.1	−54.6 ± 1.0	−31.5	−105

[a] Used concentrations: [receptor] = 0.3 mM, [sugar] = 3 mM. [b] The errors listed are the standard deviations for a minimum of three replicate ITC titrations.

An increase in binding affinity in the presence of water in organic solvents was already observed for other carbohydrate receptors.[1,19] The authors pointed out that the synthetic systems have the tendency to adopt the strategy widely used by nature and that the addition of water increases pyranoside binding "by filling in the gaps between the receptor and ligand".[19]

The microcalorimetric titrations of **12** and **13** with sugar **24** revealed the expected more favourable binding properties of **13** compared to **12**, confirmed the tendencies suggested by the results of the NMR titrations, clearly showed the strong influ-

ence of the nature of the bridge units on the binding strength and provided interesting information about the driving forces of the binding processes in dry and water-containing solvents.[20]

It is worth noting that the analysis of calorimetrically determined thermodynamic parameters for more than 60 protein–carbohydrate associations[21] revealed that the enthalpy of binding is more negative than or equal to the free energy of binding and showed strong enthalpy–entropy compensation for associations in aqueous solution. Interestingly, experiments with compounds **12** and **13** in $CDCl_3$ and water-contain-
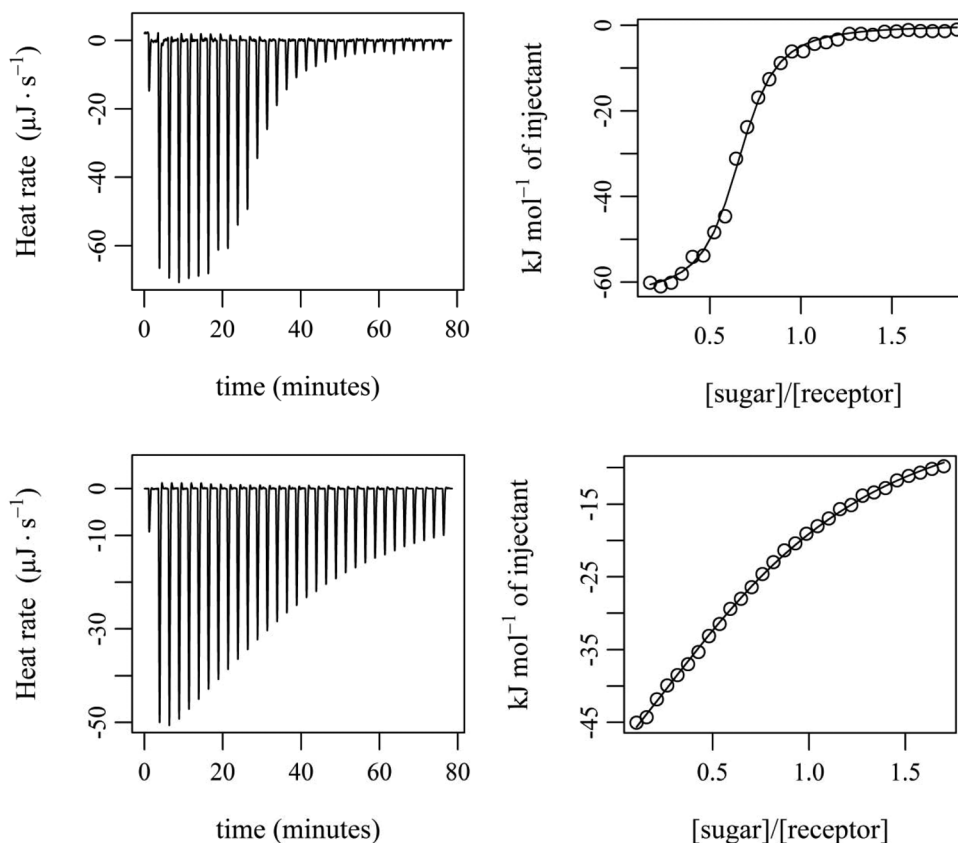
Fig. 9 (a) ITC thermogram (left) and titration curve-fitting by independent model (right) of **13** with **24** in dry $CDCl_3$. Titration mode: addition of β-glucoside ($c_{syringe}$ = 3.03 mM) into **24** (c_{cell} = 0.31 mM) at 298 K in 30 steps. (b) ITC thermogram (left) and titration curve-fitting by independent model (right) of **12** with **24** in $CDCl_3$. Titration mode: addition of β-glucoside ($c_{syringe}$ = 3.01 mM) into **12** (c_{cell} = 0.31 mM) at 298 K in 30 steps.
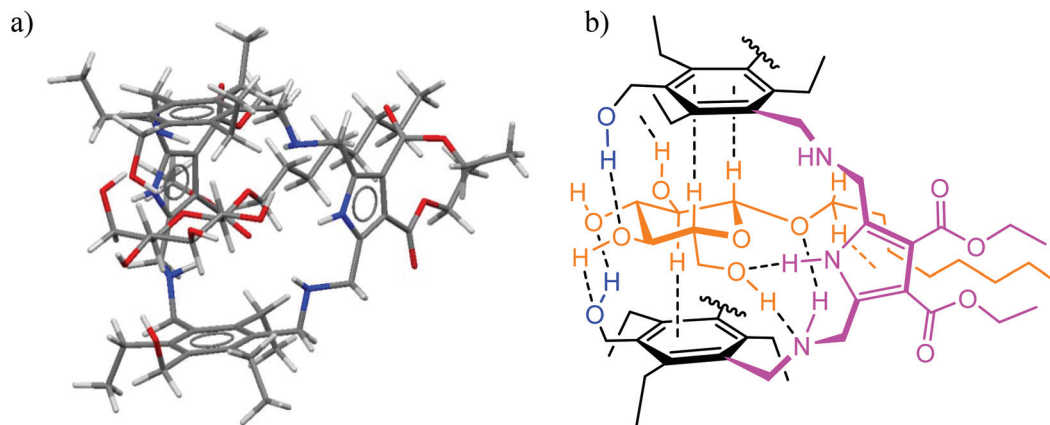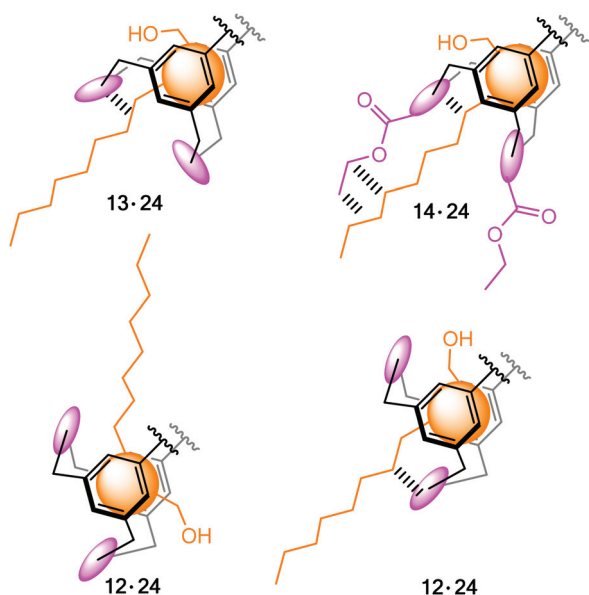
Fig. 10 (a) Energy-minimized structure of the 1 : 1 complex formed between receptor **14** and octyl β-glucoside **24** (MacroModel V.8.5, OPLS 2001 force field, MCMM, 50 000 steps). Color code: N, blue; O, red; C, gray. (b) Schematic representation of selected binding motifs indicated by molecular modelling and NMR spectroscopy for **14·24**.

ing CDCl$_3$ also show similar tendency and revealed that the enthalpy of binding is more negative than the free energy of binding.

Exemplary ROESY and molecular modelling studies

Structural aspects of binding were also analysed in detail on the basis of ROESY studies (schematic representations of some short contacts between β-glucoside **24** and receptor **13** or **14** indicated by ROESY spectra are given in the ESI†). The ROESY experiments suggested the binding modes, which were indicated by molecular modelling calculations, and gave detailed structures for the receptor–sugar complexes, as shown exem-

plarily for **14·24** in Fig. 10. As observed for the previously studied receptor–sugar complexes,[3] all OH groups of the bound sugar are involved in the formation of hydrogen bonds with **13** and **14**. Furthermore, both sides of the pyranose ring are involved in CH···π interactions with the two central triethylbenzene rings of the receptor molecule, so that the sugar is fully encapsulated in the receptor cavity. It should be noted at this point that in the complexes of sugar binding proteins often one or two aromatic groups stack on the sugar ring.[10] Interactions of the octyl chain of **24** with the pyrrole rings of **13** and **14** were also indicated by the performed studies. In the case of **12**, the formation of complexes with different orientation of the sugar molecule in the cavity of **12** was indicated and is schematic illustrated in Fig. 11.
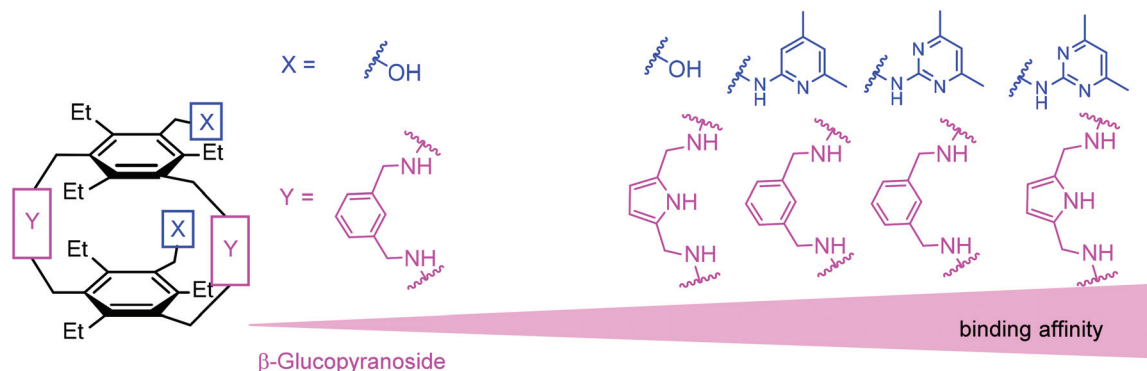
Conclusion

Our previous studies with compounds consisting of both a macrocyclic building block and two flexible side arms revealed the high potential of this receptor architecture for versatile structural modifications, which allow the recognition of important structure–activity relationships. We were now interested to see how the replacement of the heterocycle-bearing side arms, used as building blocks for the construction of the earlier receptors,[2,3] by hydroxy groups affects the binding capability of the new compounds. The use of hydroxy groups as recognition sites was inspired by the participation of the side chain hydroxy group of serine and threonine in the biorecognition of carbohydrates. New representatives of macrocyclic compounds bearing two hydroxymethyl groups as side arms were prepared and their binding properties toward selected carbohydrates were evaluated.

Compound **12**, containing benzene-based bridges, was prepared as an analogue of the earlier developed carbohydrate receptors **1** and **2**, consisting of aminopyridine- or aminopyrimidine-bearing side arms. Compounds **13** and **14**, incorporat-

Fig. 11 Schematic representation of the orientation of octyl β-glucoside **24** in the receptor cavity: complexes **12·24**, **13·24** and **14·24** (bridge units of the receptors **12–14** are marked in magenta, the sugar molecule is highlighted in orange).

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10648–10659 | **10655**

Fig. 12 Schematic illustration of the influence of the used bridge/side arm combination (Y/X combination) on the binding strength toward β-glucopyranoside in the case of compounds consisting of both a macrocyclic building block and flexible side-arms.

ing pyrrole-based bridges, were prepared as analogues of the powerful receptors **3–5**. Binding properties of the new compounds were evaluated on the basis of experiments in two-phase systems, such as phase transfer of sugars from aqueous into organic solvents, and in homogeneous organic media by performing ^{1}H NMR spectroscopic titrations, ROESY experiments and microcalorimetric titrations.

Although the replacement of aminopyridine- or aminopyrimidine-bearing side arms by hydroxy groups caused a decrease in the binding affinity, the hydroxymethyl groups used in combination with suitable bridge units were revealed to be valuable building blocks for carbohydrate receptors of this type. For instance, the combination of hydroxymethyl groups with pyrrole-bearing bridges (compounds **13**) provides a powerful receptor, having the ability to complex the tested β-glucoside with an association constant K_{11} of about 200 000 M^{-1} in CDCl$_3$. Molecular modelling calculations indicated the formation of the expected hydrogen bonds and CH–π interactions between the binding partners, the existence of which was also suggested by ROESY experiments. The replacement of the pyrrole-bearing bridges by benzene-based units significantly weakened binding by a factor of about 20. Such binding behaviour of **12** is mainly a consequence of the less favourable hydrogen bonding capabilities of the benzene-based bridges and may also be a result of the tendency of **12** to form more intramolecular interactions[22] (OH···O, NH···O and NH···π) than **13**, as indicated by molecular modeling calculations (see Fig. S6 and S7†). The microcalorimetric titrations revealed that the tested compounds form enthalpy driven complexes with sugar **24** in dry CDCl$_3$ and in a water-containing solvent and that the enthalpic driving force is partially compensated by a negative ΔS.

The present results and the previously observed binding preferences indicate that the combination of two heterocycle- or hydroxyl-bearing side arms with pyrrole-based bridges represents a particularly suitable architecture for the recognition of β-glucoside. The comparison of the binding properties of **1–5** and **12–14** revealed that for the different combinations of the side arms and bridges (combinations of X and Y units),

the affinity of the considered compounds increases in the order shown in Fig. 12.

The performed studies show once again that the binding properties of the investigated type of receptor can be usefully modified by relatively simple structural variations. Further structural modifications, with which we hope to recognise characteristic structural features that are able to affect the binding properties in a predictable[23] way, are currently in progress.

Experimental section

Analytical TLC was carried out on silica gel 60 F$_{254}$ plates; column chromatography was performed on silica gel. Melting points are uncorrected. The tested monosaccharides **22–24** are commercially available. Descriptions of the binding studies in two-phase systems (extraction experiments), ^{1}H NMR spectroscopic titrations and microcalorimetric titrations are given in the ESI.†

General procedure for the synthesis of compounds 12 and 13

To a solution of 3,5-bis(aminomethyl)-1-hydroxymethyl-2,4,6-triethylbenzene (**18**) in dry EtOH (10 mL) the corresponding aldehyde (**19** or **20**) and one drop of acetic acid were added and the resulting mixture was stirred for 7 h (in the case of **12**) or 4 h (in the case of **13**) at 45 °C. After the mixture was cooled to room temperature, the precipitate (imine **12a** or **13a**) was filtered off, washed with small amounts of EtOH and suspended in dry MeOH (4 ml) or MeOH/CH$_2$Cl$_2$ (2 : 1 v/v, 6 mL; in the case of **13**). To this solution NaBH$_4$ (5 to 10 equiv.) was slowly added and the mixture was stirred at room temperature for 4 h. Then the solvent was evaporated, the residue was suspended in a mixture of H$_2$O/CHCl$_3$ (9 : 1 v/v, 10 mL) and the suspension was stirred again for another 12 h. Afterwards, the suspension was extracted with CHCl$_3$ and the combined organic layers were washed with H$_2$O and dried over MgSO$_4$. The solvent was evaporated and the residue was dried under vacuum.

10656 | *Org. Biomol. Chem.*, 2016, **14**, 10648–10659

This journal is © The Royal Society of Chemistry 2016

Compound 12. Used amounts of starting materials: 3,5-bis(aminomethyl)-1-hydroxymethyl-2,4,6-triethylbenzene (18) (165 mg, 0.66 mmol) and isophthalaldehyde (19) (88 mg, 0.66 mmol). Yield: 86% (201 mg, 0.29 mmol). Mp 213–215 °C. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.12 (t, J = 7.4 Hz, 6H), 1.22 (t, J = 7.4 Hz, 12H), 2.67 (q, J = 7.4 Hz, 4H), 2.85 (q, J = 7.4 Hz, 8H), 3.74 (s, 8H), 3.88 (s, 8H), 4.69 (s, 4H), 7.15 (d, J = 7.3 Hz, 4H), 7.23 (m, 2H), 7.51 (s, 2H). ^{13}C NMR (125 MHz, CDCl$_3$): δ = 16.85, 17.00, 22.61, 22.63, 47.35, 54.92, 59.05, 127.02, 127.08, 127.99, 134.33, 134.50, 140.55, 142.55, 143.18. MS (ESI) calcd for $C_{46}H_{65}N_4O_2$: 705.5 [M + H]$^+$, found 705.5. HR-MS (ESI) calcd for $C_{46}H_{65}N_4O_2$: 705.510204 [M + H]$^+$, found 705.510238.

Compound 13. Used amounts of starting materials: 3,5-bis(aminomethyl)-1-hydroxymethyl-2,4,6-triethylbenzene (18) (163 mg, 0.65 mmol) and 1H-pyrrole-2,5-dicarbaldehyde (20) (80 mg, 0.65 mmol). Yield: 83% (184 mg, 0.26 mmol). Mp 157–159 °C (dec.). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.20 (t, J = 7.4 Hz, 6H), 1.25 (t, J = 7.5 Hz, 12H), 2.82 (q, J = 7.4 Hz, 4H), 2.95 (q, J = 7.5 Hz, 8H), 3.75 (s, 8H), 3.92 (s, 8H), 4.73 (s, 4H), 5.95 (d, J = 2.6 Hz, 4H), 8.83 (s, 2H). ^{13}C NMR (125 MHz, CDCl$_3$): δ = 16.76, 16.88, 22.46, 22.53, 46.91, 47.54, 58.46, 105.58, 129.71, 129.89, 133.80, 134.56, 142.47. HRMS (ESI) calcd for $C_{42}H_{63}N_6O_2$: 683.500702 [M + H]$^+$, found 683.500682.

Compound 14. 3,5-Bis(aminomethyl)-1-hydroxymethyl-2,4,6-triethylbenzene (18) (147 mg, 0.59 mmol) and diethyl 2,5-diformyl-1H-pyrrole-3,4-dicarboxylate (21) (157 mg, 0.59 mmol) were dissolved in dry dichloromethane (25 mL) and stirred at 40 °C for 17 h (in the presence of molecular sieves 4 Å). Then the CH$_2$Cl$_2$ solution was decanted, mixed with dry MeOH (4 mL) and sodium borohydride (126 mg, 3.2 mmol) was added to the reaction mixture. After 2.5 h stirring at room temperature the solvent was removed, the residue was suspended in a mixture of H$_2$O/CHCl$_3$ (9 : 1 v/v, 10 mL) and the suspension was stirred again for another 14 h. Afterwards, the suspension was extracted with CHCl$_3$, the combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography [silica gel, CHCl$_3$/CH$_3$OH (10 : 1 v/v) + 1% NH$_3$ in MeOH (7 N)]. Product 3 was obtained as a slightly yellowish solid.

Yield: 41% (118 mg, 0.12 mmol). Mp 119–121 °C. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.14 (m, 18H), 1.35 (t, J = 7.1 Hz, 12H), 2.74 (q, J = 7.3 Hz, 8H), 2.89 (q, J = 7.3 Hz, 4H), 3.72 (s, 8H), 4.04 (s, 8H), 4.29 (q, J = 7.1 Hz, 8H), 4.62 (s, 4H), 9.43 (br.s, 2H). ^{13}C NMR (125 MHz, CDCl$_3$): δ = 14.40, 16.52, 17.00, 22.31, 22.55, 45.96, 47.48, 58.61, 60.23, 112.10, 133.66, 134.78, 135.14, 142.39, 143.66, 165.07. HRMS (ESI) calcd for $C_{54}H_{79}N_6O_{10}$: 971.585219 [M + H]$^+$, found 971.585277.

1-Hydroxymethyl-3,5-bis(phthalimidomethyl)-2,4,6-triethylbenzene (17). 1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (15) (5 g, 11.33 mmol) and potassium phthalimide (16) (6.3 g, 34.01 mmol) were dissolved in 50 mL DMSO containing H$_2$O (3.5–5 mL). The reaction mixture was stirred for 17 h at 100 °C. After cooling down to room temperature the reaction mixture was poured onto 100 mL H$_2$O. The resulting precipitate was filtered off and washed with H$_2$O to remove the excessive DMSO.

Then the solid was suspended in H$_2$O and extracted with CHCl$_3$ (7 × 30 mL). The combined organic layers were dried over MgSO$_4$, the solvent was evaporated and the crude product was purified by flash column chromatography [silica gel, toluene/ethyl acetate (solvent gradient 24 : 1–2 : 1 (v/v))]. Product 17 was obtained as a white solid.

Yield: 43% (2.47 g, 4.84 mmol). Mp 129–131 °C. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 0.99 (t, J = 7.6 Hz, 3H), 1.12 (t, J = 7.5 Hz, 6H), 2.97 (q, J = 7.5 Hz, 4H), 3.17 (q, J = 7.6 Hz, 2H), 4.75 (d, J = 5.2 Hz, 2H), 4.95 (s, 4H), 7.76–7.65 (m, 4H), 7.87–7.76 (m, 4H). ^{13}C NMR (125 MHz, CDCl$_3$): δ = 15.86, 16.35, 22.92, 23.44, 37.43, 59.05, 123.25, 129.58, 131.98, 134.00, 134.73, 144.70, 145.92, 168.28. MS (ESI) calcd for $C_{31}H_{30}N_2O_5Na$: 533.20, found 533.20 [M + Na]$^+$. Elemental analysis, calcd for $C_{31}H_{30}N_2O_5$: C 72.92%, H 5.92%, N 5.49%, O 15.67%; found C 72.49%, H 6.24%, N 5.22%.

1-Hydroxymethyl-3,5-bis(aminomethyl)-2,4,6-triethylbenzene (18). 1-Hydroxymethyl-3,5-bis(phthalimidomethyl)-2,4,6-triethylbenzene (17) (451 mg, 0.88 mmol) was dissolved in a mixture of toluene/EtOH (2 : 1 v/v, 30 mL) and refluxed with hydrazine hydrate (64%, 0.18 mL) for 16 h. After cooling down to room temperature, the reaction mixture with the precipitate was stirred and a solution of 40% aq. KOH was added until the solid was completely dissolved. Then the organic layer was separated, dried over MgSO$_4$ and the solvent was removed under vacuum. 18 was obtained as a white solid.

Yield: 91% (201 mg, 0.80 mmol). Mp 108–110 °C. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.24 (m, 9H), 2.83 (q, J = 7.5 Hz, 2H), 2.87 (q, J = 7.5 Hz, 4H), 3.88 (s, 4H), 4.74 (s, 2H). ^{13}C NMR [125 MHz, CDCl$_3$/CD$_3$OD 50 : 1 (v/v)]: δ = 16.83, 22.61, 22.85, 38.99, 58.31, 135.06, 136.37, 141.51, 142.17. MS (ESI) calcd for $C_{15}H_{26}N_2O$: 250.20, found 251.15 [M + H]$^+$, 273.15 [M + Na]$^+$, 289.14 [M + K]$^+$, 501.41 [2M + H]$^+$.

Acknowledgements

References

1 M. Mazik, H. Cavga and P. G. Jones, *J. Am. Chem. Soc.*, 2005, **127**, 9045–9052.
2 J. Lippe and M. Mazik, *J. Org. Chem.*, 2013, **78**, 9013.
3 J. Lippe and M. Mazik, *J. Org. Chem.*, 2015, **80**, 1427.
4 For discussions on the importance of carbohydrate–aromatic interactions, see: (*a*) J. L. Asensio, A. Arda, F. J. Caňada and J. Jiménez-Barbero, *Acc. Chem. Res.*, 2013, **46**, 946–954; (*b*) S. Tsuzuki, T. Uchimaru and M. Mikami, *J. Phys. Chem. B*, 2009, **113**, 5617–5621; (*c*) G. Terraneo, D. Potenza, A. Canales, J. Jiménez-Barbero, K. K. Baldridge and A. Bernardi, *J. Am. Chem. Soc.*, 2007, **129**, 2890–2900; (*d*) M. I. Chávez, C. Andreu, P. Vidal, N. Aboitiz, F. Freire, P. Groves, J. L. Asensio, G. Asensio, M. Muraki, F. J. Caňada and J. Jiménez-Barbero, *Chem. – Eur. J.*, 2005, **11**, 7060–

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10648–10659 | 10657

7074; (e) J. Screen, E. C. Stanca-Kaposta, D. P. Gamblin, B. Liu, N. A. Macleod, L. C. Snoek, B. G. Davis and J. P. Simons, *Angew. Chem., Int. Ed.*, 2007, **46**, 3644–3648; (f) S. H. Kiehna, Z. R. Laughrey and M. L. Waters, *Chem. Commun.*, 2007, 4026–4028.

5 For examples of CH–π interactions in the crystal structures of complexes formed between artificial receptors and carbohydrates, see ref. 1.

6 (a) For a discussion on CH–π interactions, see: M. Nishio, *Phys. Chem. Chem. Phys.*, 2011, **13**, 13873–13900; (b) For a discussion on the importance of aromatic rings in chemical and biological recognition, see: L. M. Salonen, M. Ellermann and F. Diederich, *Angew. Chem., Int. Ed.*, 2011, **50**, 4808–4812.

7 For reviews on carbohydrate recognition with artificial receptors using noncovalent interactions, see: (a) A. P. Davis and T. D. James, in *Functional Synthetic Receptors*, ed. T. Schrader and A. D. Hamilton, Wiley-VCH, Weinheim, Germany, 2005, pp. 45–109; (b) A. P. Davis and R. S. Wareham, *Angew. Chem., Int. Ed.*, 1999, **38**, 2978–2996; (c) D. B. Walker, G. Joshi and A. P. Davis, *Cell. Mol. Life Sci.*, 2009, **66**, 3177–3191; (d) M. Mazik, *Chem. Soc. Rev.*, 2009, **38**, 935–956; (e) S. Jin, Y. Cheng, S. Reid, M. Li and B. Wang, *Med. Res. Rev.*, 2010, **30**, 171–257; (f) A. P. Davis, *Org. Biomol. Chem.*, 2009, **7**, 3629–3638; (g) S. Kubik, *Angew. Chem., Int. Ed.*, 2009, **48**, 1722–1725; (h) M. Mazik, *ChemBioChem*, 2008, **9**, 1015–1017; (i) M. Mazik, *RSC Adv.*, 2012, **2**, 2630–2642.

8 P. Adhikari, K. Bachhawat-Sikder, C. J. Thomas, R. Ravishankar, A. Arockia Jeyaprakash, V. Sharma, M. Vijayan and A. Surolia, *J. Biol. Chem.*, 2001, **276**, 40734–40739.

9 J. H. Naismith and R. A. Field, *J. Biol. Chem.*, 1996, **271**, 972–976.

10 For examples, see: (a) H. Lis and N. Sharon, *Lectins*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2003; (b) H. Lis and N. Sharon, *Chem. Rev.*, 1998, **98**, 637–674; (c) F. A. Quiocho, *Pure Appl. Chem.*, 1989, **61**, 1293–1306; (d) W. I. Weiss and K. Drickamer, *Annu. Rev. Biochem.*, 1996, **65**, 441–473; (e) H. J. Gabius, *The Sugar Code – Fundamentals of Glycoscience*, Wiley-Blackwell, 2009; (f) H.-J. Gabius, S. André, J. Jiménez-Barbero, A. Romero and D. Solis, *Trends Biochem. Sci.*, 2011, **36**, 298–313.

11 For examples of acyclic receptors bearing hydroxy groups, which we have previously studied, see: (a) M. Mazik and M. Kuschel, *Eur. J. Org. Chem.*, 2008, 1517–1526; (b) M. Mazik and A. König, *Eur. J. Org. Chem.*, 2007, 3271–3276; (c) For examples of other acyclic receptors studied by our group, see ref. 12–14.

12 For examples of benzene-based receptors bearing phenanthroline, naphthyridine, quinoline, pyridine, pyridinium, quinolinium, alkylamino or oxime groups, see: (a) C. Geffert and M. Mazik, *J. Org. Chem.*, 2013, **78**, 292–300; (b) M. Mazik and C. Geffert, *Org. Biomol. Chem.*, 2011, **9**, 2319–2326; (c) M. Mazik and C. Sonnenberg, *J. Org. Chem.*, 2010, **75**, 6416–6423; (d) M. Mazik, A. Hartmann

and P. G. Jones, *Chem. – Eur. J.*, 2009, **15**, 9147–9159; (e) M. Mazik and A. Hartmann, *J. Org. Chem.*, 2008, **73**, 7444–7450; (f) M. Mazik and H. Cavga, *Eur. J. Org. Chem.*, 2007, 3633–3363; (g) M. Mazik and H. Cavga, *J. Org. Chem.*, 2006, **71**, 2957–2963; (h) M. Mazik, M. Kuschel and W. Sicking, *Org. Lett.*, 2006, **8**, 855–858; (i) M. Mazik and W. Sicking, *Tetrahedron Lett.*, 2004, **45**, 3117–3121; (j) M. Mazik and W. Sicking, *Chem. – Eur. J.*, 2001, **7**, 664–670; (k) M. Mazik, H. Bandmann and W. Sicking, *Angew. Chem., Int. Ed.*, 2000, **39**, 551–554; (l) M. Mazik, W. Radunz and R. Boese, *J. Org. Chem.*, 2004, **69**, 7448–7462; (m) M. Mazik, W. Radunz and W. Sicking, *Org. Lett.*, 2002, **4**, 4579–4582; (n) M. Mazik and A. C. Buthe, *J. Org. Chem.*, 2007, **72**, 8319–8326; (o) M. Mazik and A. C. Buthe, *Org. Biomol. Chem.*, 2008, **6**, 1558–1568.

13 For examples of carbohydrate receptors containing imidazole, benzimidazole, indole, pyrrole, pyrazole or pyrimidine groups and 2,4,6-triethyl-, trimethyl- or trimethoxybenzene scaffold, see: (a) J.-R. Rosien, W. Seichter and M. Mazik, *Org. Biomol. Chem.*, 2013, **11**, 6569–6579; (b) M. Mazik and M. Kuschel, *Chem. – Eur. J.*, 2008, **14**, 2405–2419; (c) M. Mazik and A. Hartmann, *Beilstein J. Org. Chem.*, 2010, **6**(9), DOI: 10.3762/bjoc.6.9; (d) C. Sonnenberg, A. Hartmann and M. Mazik, *Nat. Prod. Commun.*, 2012, **7**, 321–326; (e) M. Mazik and H. Cavga, *J. Org. Chem.*, 2007, **72**, 831–838; (f) J. Lippe, W. Seichter and M. Mazik, *Org. Biomol. Chem.*, 2015, **13**, 11622; (g) N. Koch and M. Mazik, *Synthesis*, 2016, 48.

14 For examples of carbohydrate receptors consisting of a biphenyl-, diphenylmethane- or dimesitylmethane-based scaffold, see ref. 11b and: (a) M. Mazik and A. König, *J. Org. Chem.*, 2006, **71**, 7854–7857; (b) M. Mazik and A. C. Buthe, *Org. Biomol. Chem.*, 2009, **7**, 2063–2071; (c) N. Koch, J.-R. Rosien and M. Mazik, *Tetrahedron*, 2014, **70**, 8758–8767.

15 For examples of other macrocyclic carbohydrate receptors bearing pyrrole-based bridges, see: (a) G. Joshi and A. P. Davis, *Org. Biomol. Chem.*, 2012, **10**, 5760–5763; (b) O. Francesconi, M. Gentili, C. Nativi, A. Ardá, F. J. Cañada, J. Jimenez-Barbero and S. Roelens, *Chem. – Eur. J.*, 2014, **20**, 6081–6091.

16 G. Sudhakar, V. D. Kadam, S. Bayya, G. Pranitha and B. Jagadeesh, *Org. Lett.*, 2011, **13**, 5452–5455 and references therein.

17 D. T. Gryko and M. Galęzowski, *Org. Lett.*, 2005, **7**, 1749–1752.

18 (a) C. Ke, H. Destecroix, M. P. Crump and A. P. Davis, *Nat. Chem.*, 2012, **4**, 718–723; (b) N. P. Barwell and A. P. Davis, *J. Org. Chem.*, 2011, **76**, 6548–6557; (c) E. Klein, M. P. Crump and A. P. Davis, *Angew. Chem., Int. Ed.*, 2005, **44**, 298–302; (d) E. Klein, Y. Ferrand, E. K. Auty and A. P. Davis, *Chem. Commun.*, 2007, 2390–2392; (e) Y. Ferrand, M. P. Crump and A. P. Davis, *Science*, 2007, **318**, 619–622; (f) Y. Ferrand, E. Klein, N. P. Barwell, M. P. Crump, J. Jiménez-Barbero, C. Vicent, G.-J. Boons, S. Ingale and A. P. Davis, *Angew. Chem., Int. Ed.*, 2009, **48**, 1775–1779.

10658 | *Org. Biomol. Chem.*, 2016, **14**, 10648–10659

This journal is © The Royal Society of Chemistry 2016

19 (*a*) R. P. Bonar-Law and J. K. M. Sanders, *J. Am. Chem. Soc.*, 1995, **117**, 259–271; (*b*) T. Mizutami, T. Kurahashi, T. Murakami, N. Matsumi and H. Ogoshi, *J. Am. Chem. Soc.*, 1997, **119**, 8991–9001.

20 (*a*) For a recent discussion on experimental binding energies in supramolecular complexes, see: F. Biedermann and H.-J. Schneider, *Chem. Rev.*, 2016, **116**, 5216–5300; (*b*) For a recent discussion on dispersive interactions in solution complexes, see: H.-J. Schneider, *Acc. Chem. Res.*, 2015, **48**, 1815–1822.

21 E. J. Toone, *Curr. Opin. Struct. Biol.*, 1994, **4**, 719–728.

22 For an analysis of the influence of intramolecular hydrogen bonds on the binding abilities of artificial receptors, see: B. Dolenský, R. Konvalinka, M. Jakubek and V. Král, *J. Mol. Struct.*, 2013, **1035**, 124–128.

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

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10648–10659 | **10659**