ARTICLE

OBC
www.rsc.org/obc

Complexation of phenolic guests by *endo*- and *exo*-hydrogen-bonded receptors †

Jessica M. C. A. Kerckhoffs,[a] Tsutomu Ishi-i,[a,b] Vasile Paraschiv,[a] Peter Timmerman,[a] Mercedes Crego-Calama,*[a] Seiji Shinkai[c] and David N. Reinhoudt*[a]

[a] *Laboratory of Supramolecular Chemistry and Technology, MESA$^+$ Research Institute, University of Twente, P. O. Box 217, 7500 AE Enschede, The Netherlands. E-mail: m.crego-calama@ct.utwente.nl*

[b] *Institute of Advanced Material Study, Kyushu University, 6-1 Kasuga-kohen, Kasuga, 816-8586, Japan*

[c] *Chemotransfiguration Project, Japan Science and Technology Corporation (JST), 2432 Aikawa, Kurume, Fukuoka 839-0861, Japan*

Received 17th March 2003, Accepted 4th June 2003
First published as an Advance Article on the web 20th June 2003

This article describes the complexation of phenol derivatives by hydrogen-bonded receptors. These phenol receptors are formed by self-assembly of calix[4]arene dimelamine or tetramelamine derivatives with 5,5-diethylbarbiturate (DEB) or cyanurate derivatives (CYA). The double rosette assemblies $\mathbf{3}_3\cdot(\mathrm{DEB})_6/(\mathrm{CYA})_6$ have their phenol-binding functionalities (ureido groups) at the top and at the bottom of the double rosette (*exo*-receptors). The tetrarosette assemblies $\mathbf{4}_3\cdot(\mathrm{DEB})_{12}/(\mathrm{CYA})_{12}$ form a cavity with binding sites between the two double rosettes for guest encapsulation (*endo*-receptors). An intrinsic binding constant K_a of 202 M^{-1} and 286 M^{-1} for the binding of 4-nitrophenol to the ureido functionalized *exo*- and *endo*-receptors, respectively, was observed. For the *exo*-receptor a 1 : 6 stoichiometry was observed while for the *endo*-receptor 1 : 4 binding stoichiometry was determined by Job plot and MALDI-TOF MS. The important role that the hydroxy group's acidity plays in the complexation of 4-nitrophenol is clarified by binding studies with different phenol derivatives. The hydrogen-bonded receptors showed a much smaller response towards less acidic phenol derivatives.

Introduction

Molecular recognition of substrates (guests) by receptors is a topic studied intensively in (bio)chemistry.[1] Compared to natural systems, most synthetic receptors[2] described in the literature support only a limited number of weak interactions. These synthetic receptors are usually only capable of binding a single guest, be it a cation,[3] an anion,[4] or a small neutral molecule.[5] Furthermore, the labor-intensive synthesis of these covalent receptors has been for decades based on rational design, where the focus is on optimization of the electronic and steric complementarity between the synthetic host and the targeted guest molecule.[1] However, natural receptors can selectively bind much larger and more structurally complex substrates than smaller synthetic receptors. This is possible because natural receptors use multiple interactions to enhance the binding strength,[6] by simple combinations of the basic set of natural "tools" such as amino acids and nucleotides.[7] In proteins, the binding strength of a molecule to a target is increased when the target displays multiple sites for binding and the protein contains several discrete binding sites.[8]

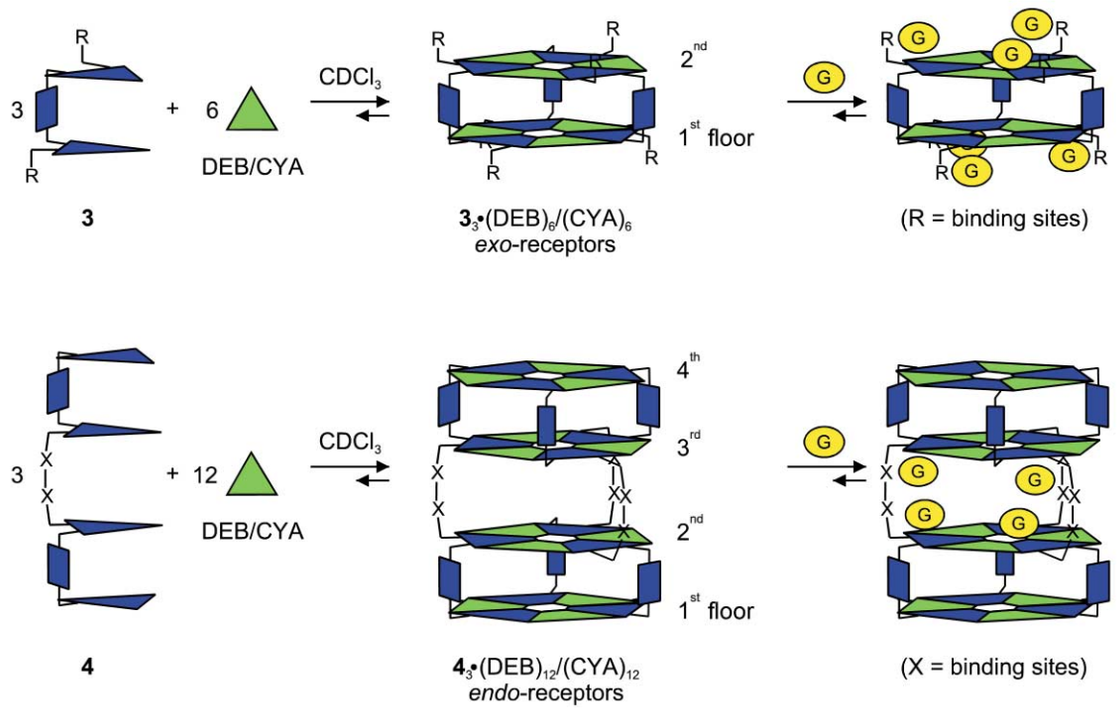
In attempts to mimic nature, interest is now focused more on the generation of diverse synthetic multivalent receptors (and ligands) rather than on their rational design.[9] For example, Still and co-workers[10] have reported the synthesis of 10^4 receptors for *N*-acylated[5]Leu enkephalin methyl ester from an A,B-*cis* cholic core by variations in the tripeptidic arms. Also, recently our group showed that the binding affinity for caffeine in water varies significantly upon the random combination of the side chains of a tetracationic porphyrin receptor.[11] Hamilton *et al.*[12] showed a nice example of functional diversity in developing a multivalent calix[4]arene based ligand bearing four peptidic loops that is able to bind to the surface of cytochrome c. Although the covalent approach has led to the synthesis of many effective receptors, we[13] and others[14,15] have recently focused effort on the creation of diversity in receptor molecules by using noncovalent synthesis. Noncovalent synthesis allows chemical diversity to be achieved much more readily by the simple combination of molecular building blocks.

We have previously reported the noncovalent synthesis of a great variety of hydrogen-bonded double[16] and tetrarosettes,[17] consisting of 9 and 15 different substituted building blocks held together by 36 and 72 cooperative hydrogen bonds, respectively (Scheme 1). The assemblies are formed by mixing calix[4]arene dimelamines with 2 or 4 equivalents of DEB (5,5-diethyl-barbiturate) or CYA (cyanurates derivatives) in apolar solvents. These rosette assemblies are used as a simple platform to bring together multiple ligand binding sites, that are attached to the melamine units. The approach can be used even with polar hydrogen bond donor and acceptor groups, such as small peptides.[18] The main advantage of this noncovalent approach is that a library of potential receptors can be easily generated in solution by simply mixing different calix[4]arene dimelamines with 2 or 4 equivalents of DEB or CYA under conditions that allow for the reversible exchange of the melamine derivatives.[13]

These noncovalent receptors are mainly designed for the complexation of a single guest, here and in view of our objective to extend the use of noncovalent synthesis for the generation of multivalent receptors,[19] we present our results on the complexation ability of the functionalized double and tetrarosette assemblies as *exo*- and *endo*-receptors for multiple phenolic guests, respectively. Our interest in phenol moieties resides in the fact that they are important parts of different biomolecules such as adrenergic receptors[20] and flavones.[21] Furthermore, phenols have been widely studied in physical-organic studies

† Electronic supplementary information (ESI) available: Experimental procedures, distribution of different complexes $\mathbf{3b}_3(\mathrm{DEB})_6(\mathbf{5a})_n$, MALDI-TOF data for $\mathbf{3b}_3(\mathrm{DEB})_6$ + **5a**, 1 : 6 binding model, Job plot, titration plots. See http://www.rsc.org/suppdata/ob/b3/b302836d/

DOI: 10.1039/b302836d



Scheme 1 Formation of *exo*- and *endo*-hydrogen-bonded receptors $\mathbf{3}_3\cdot(DEB)_6/(CYA)_6$ and $\mathbf{4}_3\cdot(DEB)_{12}/(CYA)_{12}$.

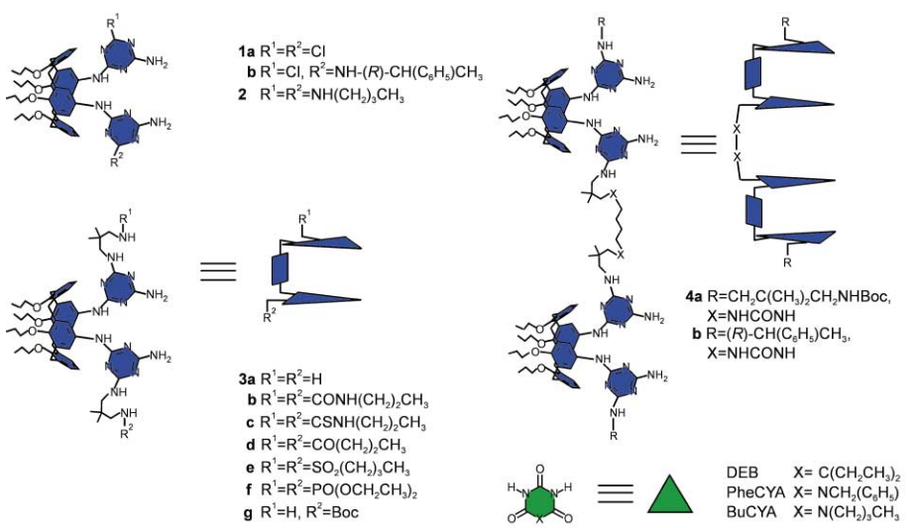


Chart 1

and for their binding to natural receptors,[22] and are therefore excellent model compounds. Despite their importance for biochemical purposes, there are not many examples of synthetic receptors for phenolic guests and to the best of our knowledge, there are not examples of noncovalent synthetic receptors for this type of guest.[23]

Results and discussion

Assemblies $\mathbf{3}_3\cdot(DEB)_6/(CYA)_6$ are *exo*-receptors in which guest recognition occurs at the bottom of the first floor and at the top of the second floor. Assemblies $\mathbf{4}_3\cdot(DEB)_{12}/(CYA)_{12}$ can be seen as *endo*-receptors in which recognition occurs in the cavity between the two double rosettes (between the second and third floors) (Scheme 1).[17]

In the double- $\mathbf{3}_3\cdot(DEB)_6/(CYA)_6$, and tetrarosettes $\mathbf{4}_3\cdot(DEB)_{12}/(CYA)_{12}$, the multiple phenol binding sites are brought together by simply mixing the corresponding building blocks in apolar solvent, such as chloroform or toluene.[18] (Scheme 1). Specifically, *exo*-receptors $\mathbf{3}_3\cdot(DEB)_6/(CYA)_6$ are formed by mixing the corresponding calix[4]arene dimelamine **3** bearing the phenol recognition sites R, with 2 equivalents of DEB or CYA. Similarly, *endo*-receptors $\mathbf{4}_3\cdot(DEB)_{12}/(CYA)_{12}$ are formed by mixing the corresponding tetramelamine **4** (containing two calix[4]arene dimelamine units covalently connected by a flexible linker bearing the phenol binding sites X) with 4 equivalents of DEB or CYA. These assemblies have a high thermodynamic stability due to the formation of 36 (double rosette) and 72 (tetrarosette) cooperative hydrogen bonds, which renders these assemblies stable in $CDCl_3$ even at concentrations of 10^{-4} M.

1. Synthesis of double- and tetrarosette assemblies

Calix[4]arene dimelamines **2**[16b] and **3a**[18] (Chart 1) were synthesized by reaction of bis(chlorotriazine) **1a** with an excess of butylamine and 2,2-dimethyl-1,3-diaminopropane, respectively. Compounds **3b–f**[18] (for detailed synthesis of **3c**, see ESI †) were synthesized by reaction of **3a** with the corresponding isocyanate or acid chloride derivative. Calix[4]arene tetramelamine **4a**[17b] (Chart 1) was synthesized by reaction of **3g**[17b] with 0.5 equiv. of 1,4-diaminobutane bis(*p*-nitrophenyl dicarbamate). Compound **4b**[17b] was synthesized by reaction of **1b** first

with an excess of 2,2-dimethyl-1,3-diaminopropane, followed by reaction of the intermediate with 0.5 equiv. of 1,4-diaminobutane bis(*p*-nitrophenyl dicarbamate). All compounds were fully characterized by ^{1}H NMR, FAB MS, and elemental analysis or ^{13}C NMR.

2. Complexation studies with *exo*-receptors

The detailed structural analysis of the assembly **3b**$_3$·(DEB)$_6$ by ^{1}H NMR spectroscopy showed that the 2,2-dimethylpropyl side chain adopts a rigid conformation. In the assembly this ureido side chain is folded back over the calix[4]arene aromatic rings due to hydrogen bond formation between one of the urea NH protons (H^m) and one of the nitrogen atoms of the triazine ring (Fig. 1).[18] This folding back of the ureido side chains results in the formation of potential binding pockets for the phenol guests at the top and bottom of the double rosette.

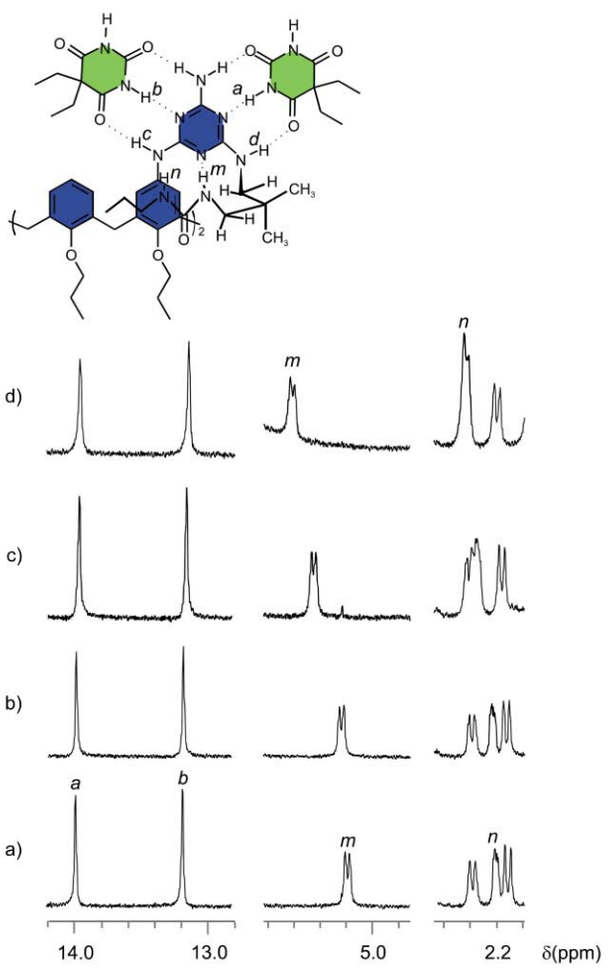


Fig. 1 Parts of the ^{1}H NMR spectra of **3b**$_3$·(DEB)$_6$ (1 mM) in $CDCl_3$ at 293K: (a) 0, (b) 1, (c) 10, and (d) 60 equivalents of **5a**.

Complexation of 4-nitrophenol (**5a**) (Chart 2) by the double rosette assembly **3b**$_3$·(DEB)$_6$ (in $CDCl_3$ at 293 K) was studied by ^{1}H NMR spectroscopy. Upon addition of **5a**, (*ca*. 60 equiv.) to **3b**$_3$·(DEB)$_6$ (1 mM), a downfield shift of ~0.45 ppm of one of the two ureido protons (H^m) was observed (Fig. 1), while the downfield shift of the other ureido proton (H^n) was smaller but still significant (~0.2 ppm). The shifts of the other rosette signals were much smaller (<0.08 ppm). Furthermore, upon addition of 1 equiv. of **5a** to **3b**$_3$·(DEB)$_6$, the ArH signal (ArH next to Ar–NO_2) of this guest showed an upfield shift of 0.085 ppm. This upfield shift is probably due to the expected formation of the hydrogen bond between the hydroxy group of **5a** and the urea carbonyls of **3b**$_3$·(DEB)$_6$.

As a control experiment, 7 equiv. of 4-nitroanisole (**6**) were added to assembly **3b**$_3$·(DEB)$_6$ (1 mM). No significant changes

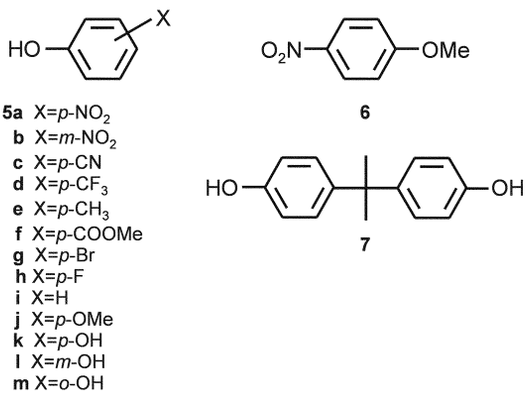


Chart 2

in the ^{1}H NMR spectrum of assembly **3b**$_3$·(DEB)$_6$ were observed. This experiment indicates that the hydroxy group of **5a** is important for the binding of **5a** to **3b**$_3$·(DEB)$_6$, supporting the hypothesis that the complexation occurs *via* the hydrogen bond formation between the carbonyl of the ureido functionality of **3b** and the hydroxy of the phenol **5a**.

The binding affinity of **5a** to the assembly **3b**$_3$·(DEB)$_6$ was determined by a ^{1}H NMR titration experiment in $CDCl_3$ (1 mM, 293 K). The chemical shift of the urea NH proton H^m was monitored during the titration (Fig. 2). Several binding models for the binding of **5a** to **3b**$_3$·(DEB)$_6$ can be envisioned. Two binding models will be discussed in detail: (i) a 1 : 2 (**3b**$_3$·(DEB)$_6$: **5a**) binding model, where phenol complexation occurs in the binding pockets above and beneath the double rosette, and (ii) a multivalent 1 : 6 binding model due to the presence of the 6 ureido groups in the assembly (3 at the top and 3 at the bottom of the double rosette). An intrinsic K_a of 134 M^{-1} was estimated by using a non-cooperative 1 : 2 binding model, and an intrinsic K_a of 202 M^{-1} was estimated by using a non-cooperative 1 : 6 binding model (Table 1).[24] The calculated K_a are on the same order as reported K_a values for the complexation of phenol derivarives to urea carbonyl groups.[25] Thus, no significant cooperativity is observed for the complexation of **5a** to **3b**$_3$·(DEB)$_6$. Nevertheless, assembly **3b**$_3$·(DEB)$_6$ has an advantage when compared with the phenol complexation to a single urea molecule. Assembly **3b**$_3$·(DEB)$_6$ has six independent binding sites that are well oriented and preorganized. They are arranged in such a way that they might bind to adjacent sites of larger guest molecules.[8a]

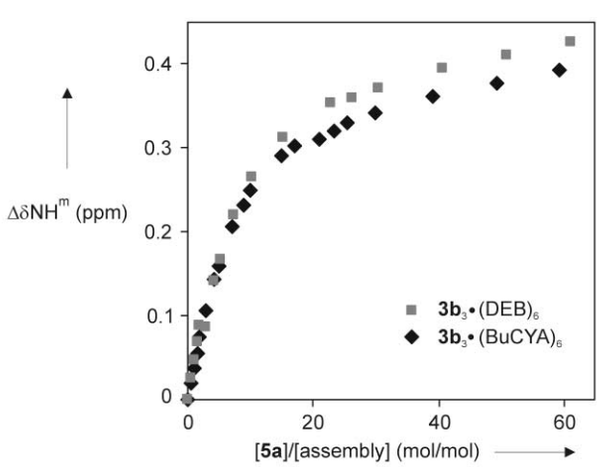


Fig. 2 Plot of the induced shifts ($\Delta\delta$NH^m) of the ^{1}H NMR signal NH^m *versus* [**5a**]/[**3b**$_3$·(DEB)$_6$] (■) and *versus* [**5a**]/[**3b**$_3$·(BuCYA)$_6$] (◆) in $CDCl_3$ (1 mM for assembly).

Further conformation of the complex stoichiometry was provided by Ag^+ labeling MALDI-TOF mass spectrometry.[26] This is a relatively soft ionization method that provides a non-

Table 1 Intrinsic binding constants (K_a)[a] for the complexations of double and tetrarosette assemblies (1 mM) with **5a** in $CDCl_3$ at 293 K[b]

Assembly	K_a/M^{-1}	
	1 : 4 Model	1 : 6 Model
$\mathbf{3b}_3\cdot(DEB)_6$	—	202 ± 17 (195 ± 15)[c]
$\mathbf{3b}_3\cdot(BuCYA)_6$	—	239 ± 14
$\mathbf{3d}_3\cdot(DEB)_6$	—	155 ± 23
$\mathbf{4a}_3\cdot(DEB)_{12}$	286 ± 19	—
$\mathbf{4a}_3\cdot(BuCYA)_{12}$	116 ± 23	—
$\mathbf{4b}_3\cdot(DEB)_{12}$	299[d] ± 16	—
$\mathbf{4b}_3\cdot(BuCYA)_{12}$	145 ± 20	—

[a] Numbers of binding constants are not measured precisely. [b] K_a values were estimated by nonlinear least-squares methods using non-cooperative 1 : 4 and 1 : 6 complex models. [c] Compound **5c** was used as a guest molecule. [d] Ref 36.

destructive way to generate charged species for the estimation of the stoichiometry of the complex. In carrying out the MALDI-TOF experiments, we used different concentrations of rosette assembly, ranging from 10 mM to 0.1 mM of $\mathbf{3b}_3\cdot(DEB)_6$, and different equivalents of phenol guest **5a** (10, 30, and 60 equiv.).[27] As shown in Fig. 3a, the mass spectrum of the sample containing $\mathbf{3b}_3\cdot(DEB)_6$ (10 mM) and **5a** (10 equiv.) showed a relatively intense signal at *m*/*z* 5589.2 (calcd for $\mathbf{3b}_3\cdot(DEB)_6\cdot(\mathbf{5a})_6\cdot Ag^+$ = 5587.8) and a smaller signal at *m*/*z* 5452 (calcd for $\mathbf{3b}_3\cdot(DEB)_6\cdot(\mathbf{5a})_5\cdot Ag^+$ = 5448.8). The mass spectrum of the sample containing 1.0 mM of the complex and 10 equiv. of **5a** showed two signals of similar intensity at *m*/*z* 5310 (calcd for $\mathbf{3b}_3\cdot(DEB)_6\cdot(\mathbf{5a})_4\cdot Ag^+$ = 5309.7) and *m*/*z* 5171 (calcd for $\mathbf{3b}_3\cdot(DEB)_6\cdot(\mathbf{5a})_3\cdot Ag^+$ = 5170.7). Also, among other signals, three smaller signals at *m*/*z* 5034, 5451, and 5588 corresponding to the complexation of 2, 5, and 6 equiv. of **5a**, respectively, were present. The mass spectrum of the most dilute sample (0.1 mM, 10 equiv. **5a**) showed a relatively intense signal at *m*/*z* 4894 (calcd for $\mathbf{3b}_3\cdot(DEB)_6\cdot\mathbf{5a}\cdot Ag^+$ = 4892.6) and smaller signals at *m*/*z* 4753 and 5033, corresponding to the free host ($\mathbf{3b}_3\cdot(DEB)_6\cdot Ag^+$) and the complexation of 2 molecules **5a** ($\mathbf{3b}_3\cdot(DEB)_6\cdot(\mathbf{5a})_2\cdot Ag^+$) respectively (Fig. 3b). In summary, these results showed that at higher concentrations of double rosette more guest molecules are complexed. The distribution of the different complexes found in the MALDI-TOF MS experiments is in agreement with the expectations based on the non-cooperative 1 : 6 binding model using a K_a of 202 M^{-1} obtained experimentally from the ^1H NMR titration (see ESI Fig 1 †). Furthermore, we have also performed MALDI-TOF MS experiments where the concentration of $\mathbf{3b}_3\cdot(DEB)_6$ was kept constant and the number of equivalents of phenol **5a** (30 and 60 equiv.) was increased. In these experiments, formation of the higher molecular weight complexes were observed upon addition of more equivalents of **5a** (see ESI Fig 2 †).

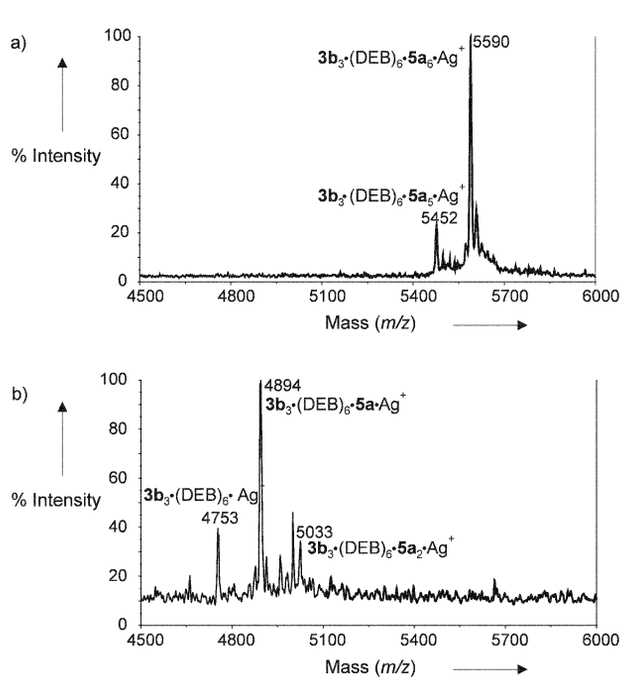


Fig. 3 MALDI-TOF mass spectra by using a Ag^+-labelling technique for the complexations of (a) $\mathbf{3b}_3\cdot(DEB)_6$ with **5a** (10 mM, 10 equiv. **5a**), and (b) $\mathbf{3b}_3\cdot(DEB)_6$ with **5a** (0.1 mM, 10 equiv. **5a**), in CH_2Cl_2.

To determine the effect of a possible change in the geometry of the complex, due to the substitution of CYA for DEB, on the complexation of phenol **5a**, the complexation of **5a** to assembly $\mathbf{3b}_3\cdot(BuCYA)_6$ was also studied by ^1H NMR. The geometry of the double rosette plane is different when DEB or CYA are part of the assembly due to the different electron distributions. We also observed a downfield shift of H^m after addition of **5a** to $\mathbf{3b}_3\cdot(BuCYA)_6$ ($\Delta\delta$ 0.25 ppm, 10 equiv. **5a**). The data obtained from the titration (1 mM rosette in $CDCl_3$, 293 K) were fitted using the 1 : 6 binding model described above to give an intrinsic K_a of 239 M^{-1}. Thus, no significant difference between the complexation of **5a** to $\mathbf{3b}_3\cdot(DEB)_6$ or to $\mathbf{3b}_3\cdot(BuCYA)_6$ was observed. This result showed that the effect of the geometry of the rosette plane on the complexation is minimal.

So far, we have presented data indicating that hydrogen bond formation between the hydroxy group of the guest and the ureido carbonyl groups of the receptor could be the driving force for phenol complexation by $\mathbf{3b}_3\cdot(DEB)_6$. To confirm the importance of this ureido side chain in promoting phenol recognition by the assembly, the interaction of **5a** with $\mathbf{2}_3\cdot(DEB)_6$, an assembly that lacks the ureido groups, was studied. A ^1H NMR titration of double rosette $\mathbf{2}_3\cdot(DEB)_6$ (1 mM, 293 K) with **5a** showed no significant changes in the chemical shifts for the ^1H NMR signals of either **5a** or $\mathbf{2}_3\cdot(DEB)_6$. Because assembly $\mathbf{2}_3\cdot(DEB)_6$ lacks the ureido protons H^m, one of the aromatic signals of **5a** (ArH next to Ar–NO_2) and proton H^d (one of the NH protons of the melamine unit, see Fig. 1 for similar description) were monitored to compare $\mathbf{3b}_3\cdot(DEB)_6$ and $\mathbf{2}_3\cdot(DEB)_6$. No significant change in the chemical shift of the aromatic protons of **5a** is observed upon addition to $\mathbf{2}_3\cdot(DEB)_6$, while in the case of the assembly of $\mathbf{3b}_3\cdot(DEB)_6$ a $\Delta\delta$ value of −0.085 ppm was observed. Also when proton H^d is followed, only small shifts (<0.025 ppm upfield) are visible during the titration with $\mathbf{2}_3\cdot(DEB)_6$, while larger shifts ($\Delta\delta$ 0.05 ppm, 10 equiv. **5a**) were observed in the case of $\mathbf{3b}_3\cdot(DEB)_6$.

Furthermore, the intensities of the diagnostic NH_{DEB}-protons, H^a and H^b (see Fig. 1 for similar H description), for the assembly of $\mathbf{2}_3\cdot(DEB)_6$ decrease after addition of 10 equiv. of phenol **5a**, suggesting that assembly dissociation occurs upon phenol addition.[28] The dissociation as revealed by ^1H NMR spectroscopy is accompanied by a color change of the solution from colorless to yellow, consistent with deprotonation of phenol **5a**.[29] In sharp contrast, when up to 60 equiv. of **5a** are added to $\mathbf{3b}_3\cdot(DEB)_6$, no color change or change in the ^1H NMR of the H^a or H^b signals is observed, indicating that dissociation of the assembly does not occur. Therefore, it seems likely that assembly $\mathbf{2}_3\cdot(DEB)_6$ is less stable and dissociates more readily than $\mathbf{3b}_3\cdot(DEB)_6$, thereby liberating the free calix[4]arene dimelamine in solution, which can then deprotonate **5a**.

We have also studied the complexation of **5a** with different functionalized double rosettes. Addition of **5a** to the thio ureido assembly ($\mathbf{3c}_3\cdot(DEB)_6$) showed only a small shift of H^m ($\Delta\delta$ 0.06 ppm, 10 equiv.) This observed shift is much smaller than the shift of H^m for the assembly of $\mathbf{3b}_3\cdot(DEB)_6$ ($\Delta\delta$ 0.26 ppm) upon addition of 10 equiv. of **5a**. Addition of ~10 equiv. of **5a** to $\mathbf{3e}_3\cdot(DEB)_6$ (sulfonamide) and $\mathbf{3f}_3\cdot(DEB)_6$ (phosphoramide) did not shift any signals in the ^1H NMR spectrum. The NMR data suggest that there is no complexation of **5a** by $\mathbf{3e}_3\cdot(DEB)_6$ or $\mathbf{3f}_3\cdot(DEB)_6$. This could be because the hydrogen

bond acceptor ability (or electron density) of the phosphoramide and sulfonamide oxygens and the sulfur of the thiourea[30] is too low to enable detectable complexation by phenol **5a**. Addition of **5a** to assembly **3d**$_3$·(DEB)$_6$, containing an amide in the side chain, showed a similar shift ($\Delta\delta$ 0.25 ppm, 10 equiv.) as was seen for ureido-containing assembly **3b**$_3$·(DEB)$_6$. The ^{1}H NMR titration of the addition of **5a** to assembly **3d**$_3$·(DEB)$_6$ yielded an intrinsic K_a of 155 M^{-1}, a value that is on the same order of magnitude as the K_a for binding of **5a** by **3b**$_3$·(DEB)$_6$.

3. Complexation studies with *endo*-receptors

To assess the difference between *exo*- and *endo*-receptors, recognition studies of **5a** by tetrarosette assemblies **4a**$_3$·(DEB)$_{12}$ were performed under the same experimental conditions as for **3b**$_3$·(DEB)$_6$. For **4a**$_3$·(DEB)$_{12}$, the urea NH$^{\alpha}$ proton signal at 5.20 ppm (Fig. 4) also showed a significant downfield shift upon addition of **5a** ($\Delta\delta$ 0.375 ppm, 10 equiv. **5a**). On the other hand, the induced shift of the NH$^{\beta}$ signal for the terminal carbamate moiety is much smaller ($\Delta\delta$ 0.02 ppm, 10 equiv. **5a**). These results indicate that the hydroxy group of **5a** interacts predominantly with the internal ureido carbonyl groups that are positioned within the cavity formed by **4a**$_3$·(DEB)$_{12}$.[31]

To demonstrate that the phenol guest complexation occurs in the cavity between the two double rosettes, the signals of the H^a and H^b protons were monitored in a typical NMR titration experiment (Fig. 4). Interestingly, upon addition of 10 equiv. of **5a** the only significant shifts were for NH^{a2} ($\Delta\delta$ −0.095 ppm) and NH^{b2} signals ($\Delta\delta$ −0.05 ppm) of the second and third floors of the tetrarosette. In contrast, the corresponding guest-induced shifts for the first and fourth rosette floors were much smaller ($\Delta\delta$ +0.01 ppm for NH^{a1} and −0.02 ppm for NH^{b1}, 10 equiv. **5a**). These data clearly indicate that phenol **5a** is complexed within the assembly's cavity between the second and the third floors of the tetrarosette. Similar results were also found for **4a**$_3$·(BuCYA)$_{12}$, **4b**$_3$·(DEB)$_{12}$, and **4b**$_3$·(BuCYA)$_{12}$ assemblies. This is to be expected since the ureido carbonyls in the linker are positioned between the second and third floors.

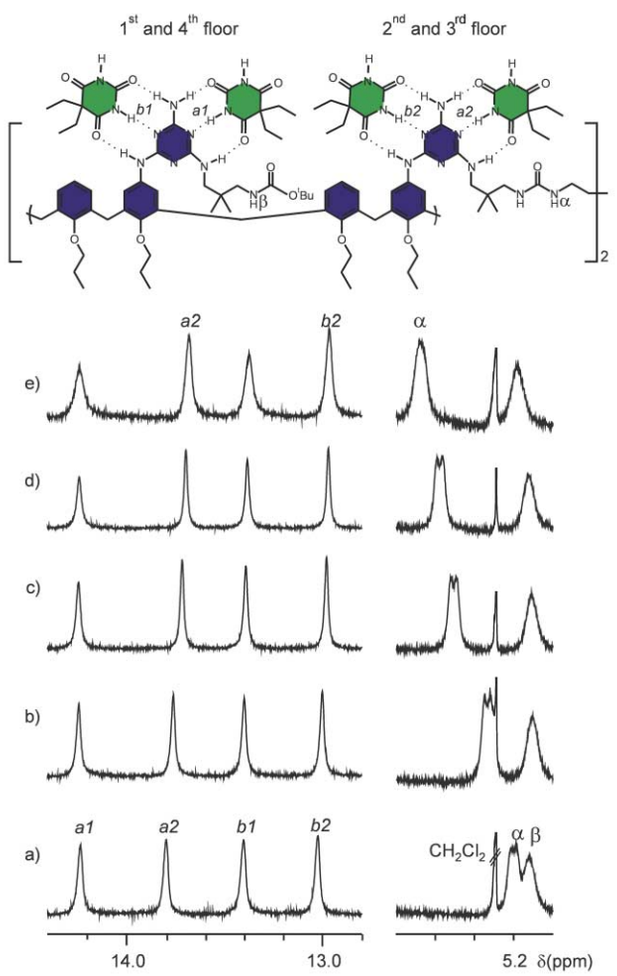


Fig. 4 Parts of the ^{1}H NMR spectra of **4a**$_3$·(DEB)$_{12}$ (1 mM) in $CDCl_3$ at 293 K: (a) 0, (b) 2, (c) 6, (d) 10, and (e) 20 equivalents of **5a**.

Generally, for barbituric acid-based assemblies (**4a**$_3$·(DEB)$_{12}$ and **4b**$_3$·(DEB)$_{12}$) the NH^{a1} and NH^{b1} peaks on the first and fourth floors tended to broaden upon the addition of **5a** (Fig. 4). The peak broadening is ascribed to proton exchange between the OH of **5a** and the NH^{a1}/NH^{b1}.[32] The peak broadening, indicative of significant proton exchange in barbituric acid-based chiral assembly **4b**$_3$·(DEB)$_{12}$, is consistent with the lower stability of this DEB-based assembly. Additional proof of the lability of the tetrarosette assembly **4b**$_3$·(DEB)$_{12}$ was gathered by the disassociation of the assembly upon the addition of excess **5a**.[33] The more stable cyanuric acid-based[34] assembly **4b**$_3$·(BuCYA)$_{12}$ did not show broadening of the NH^{a1} and NH^{b1} signals, indicating inhibition of the amide NH–phenol OH proton exchange due to the larger stability of the rosette's hydrogen bonding network.[34]

In contrast to the double rosette system, a 1 : 4 stoichiometry of the tetrarosette **4a**$_3$·(DEB)$_{12}$ complexation with **5a** was estimated. A Job plot[35] indicates a maximum at a 1 : 4 molar ratio of **4a**$_3$·(DEB)$_{12}$ and **5a** (see ESI Fig 4 †). Only four **5a** guest molecules could be complexed within the cavity[31] rather than six, probably due to space limitations. The intrinsic K_a was estimated from ^{1}H NMR titration data (see ESI Fig 5 †) by using a non-cooperative 1 : 4 complex model. The estimated values are summarized in Table 1.

Guest encapsulation in tetrarosette assemblies was also supported by MALDI-TOF mass spectrometry using the Ag$^+$-labeling technique. For **4b**$_3$·(DEB)$_{12}$, in the presence of 12 equiv. of **5a**, the ion peak for the parent assembly **4b**$_3$·(DEB)$_{12}$ (calcd. for **4b**$_3$·(DEB)$_{12}$·Ag$^+$ = 8913.7) was observed as the major species. Under the same experimental conditions **4b**$_3$·(BuCYA)$_{12}$ afforded the ion peaks for the 1 : 2 and 1 : 3 complexes with **5a** (calcd. for **4b**$_3$·(BuCYA)$_{12}$·(**5a**)$_2$·Ag$^+$ = 9203.7 and **4b**$_3$·(BuCYA)$_{12}$·(**5a**)$_3$·Ag$^+$ = 9342.8, respectively). No peak of the parent assembly **4b**$_3$·(BuCYA)$_{12}$ was observed (calcd. for **4b**$_3$·(BuCYA)$_{12}$·Ag$^+$ = 8925.7). More interestingly, upon the addition of a large excess of **5a** (45 equiv.) the 1 : 4 complex (cald. for **4b**$_3$·(BuCYA)$_{12}$·(**5a**)$_4$·Ag$^+$ = 9481.8) was detected as the main species, a stoichiometry that coincides with the results from the Job plot.

Generally, the K_a values (286–299 M^{-1}) for the barbituric acid-based assembly **4**$_3$·(DEB)$_{12}$ systems are larger compared to those for the cyanuric acid-based tetrarosettes **4**$_3$·(BuCYA)$_{12}$ (116–150 M^{-1}).[36] This observed difference in phenol binding strengths may be ascribed to structural differences between barbituric- and cyanuric acid-based assemblies. Somehow, the structure of DEB, with its tetrahedral sp^3-carbon in the 6-membered ring in host **4**$_3$·(DEB)$_{12}$ shows better complementarity for guest **5a** than host **4**$_3$·(BuCYA)$_{12}$, carrying CYA with its flatter sp^3–nitrogen. In contrast, the K_a values obtained for the complexation of **5a** to *exo*-receptors **3b**$_3$·(DEB)$_6$ and **3b**$_3$·(BuCYA)$_6$ are similar (see above), providing another indication that binding of **5a** to assembly **4a**$_3$·(DEB)$_{12}$ does not occur on the outer rosette platforms (1st and 4th floors).

VT ^{1}H NMR spectroscopy. Strong evidence for the encapsulation of guest **5a** within floors 2 and 3 of tetrarosette **4a**$_3$·(DEB)$_{12}$ was gathered from variable temperature (VT) NMR measurements. For tetrarosette assembly **4a**$_3$·(DEB)$_{12}$, splittings of the ^{1}H NMR signals H^{a1}, H^{a2}, H^{b1}, H^{b2}, and NH^a were observed in the presence of **5a** (3 and 10 equiv.) at low temperature (<273 K, Fig. 5). This signal splitting is ascribed to the presence of more than one species in solution on the NMR time-scale, namely the different complexed **4a**$_3$·(DEB)$_{12}$·**5a**$_n$ (n =

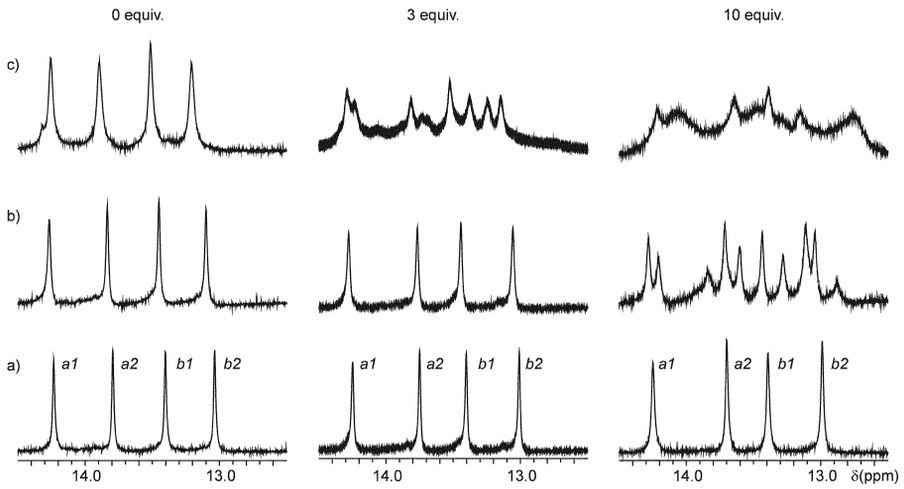


Fig. 5 Parts of the ^{1}H NMR spectra of **4a**$_3$·(DEB)$_{12}$ (1 mM) in $CDCl_3$ in the presence of **5a** (0, 3, and 10 equiv.): (a) 293, (b) 273, and (c) 233 K (For assignment, see structure in Fig. 4).

Table 2 Changes of chemical shifts ($\Delta\delta$) for the complexation of guest molecules to **3b**$_3$·(DEB)$_6$ (1 mM, $CDCl_3$ at 293 K)

Guest	pK_a [a]	σ_p/σ_m [b]	$\Delta\delta$ (ppm)	Equiv.
5a	7.15	0.81	0.26	10
5b	8.28	0.71	0.24	10
5c	7.97	0.70	0.22	10
5d		0.53	0.15	10
5e	10.17	−0.14	0.06	14
5f		0.44	0.15	10
5g		0.26	0.11	10
5i	9.89	0	0.06	10
5j		−0.28	0.05	10
5k		−0.38	0.05	5
5l	9.81	0.13	0.07	8
5m	9.85		0.19	10
6			0.0	7
7			0.16	10

[a] Values were obtained from D. R. Lide in *Handbook of Chemistry and Physics*, 83rd ed., CRC press LLC, 2002, p. 8-46–8-56. [b] Values were obtained from ref 37.

1–4) and uncomplexed **4a**$_3$·(DEB)$_{12}$ assemblies in solution. In the absence of phenol guest **5a** no splitting of the NMR signals is found (Fig. 5). Similar phenomena were also observed in **4a**$_3$·(BuCYA)$_{12}$ and **4b**$_3$·(DEB)$_{12}$ systems. In contrast to these VT results with the tetrarosette the VT ^{1}H NMR spectra of **3b**$_3$·(DEB)$_6$ with either 3 or 10 equiv. of **5a** (between 243 K and 333 K) did not show splitting of the H^a and H^b signals. For the double rosette fast exchange between the complexed guest and the free guest occurs at an even lower temperature (243 K), and only averaged NMR signals are observed.

4. Selectivity studies with *exo*- and *endo*-receptors

Various substituted-phenol derivatives **5** (Chart 2) were selected as possible guest molecules and the induced shifts of the NH$^{m/a}$ signal ($\Delta\delta$NH$^{m/a}$) for **3b**$_3$·(DEB)$_6$ (Table 2) and **4a**$_3$·(DEB)$_{12}$ were carefully checked. The $\Delta\delta$NH$^{m/a}$ values (in experiments with 1.0 mM and 10 equiv. of **5**) were plotted against Hammett parameters σ_p or σ_m (Fig. 6).[37] The σ_p or σ_m parameters are quantified descriptors of the ability of an organic functional group to affect the electron density distribution of an aryl group. While Hammett plots have been routinely used in physical-organic chemistry, they have also found increasing use in supramolecular systems.[38]

For both assemblies, the Hammett plot showed larger ^{1}H NMR chemical shift changes upon addition of more acidic phenols with stronger electron withdrawing groups, like NO_2 (**5a**), CF_3 (**5d**) and COOMe (**5f**). These results indicate, once again, the importance of the highly acidic OH group in interacting with the urea carbonyl groups in **3b**$_3$·(DEB)$_6$ and **4a**$_3$·(DEB)$_{12}$. Furthermore, for assembly **3b**$_3$·(DEB)$_6$ a good linear correlation between $\Delta\delta$NH^m and σ_p/σ_m was observed for **5a**, **5b**, **5g**, **5h**, and **5i** (bearing *p*-NO_2, *m*-NO_2, *p*-Br, *p*-F, and H respectively). For **5j** (bearing an electron donor group *p*-OMe) the observed shift is larger than expected based on the Hammett parameter σ_p, suggesting that the OMe-group may interact positively with **3b**$_3$·(DEB)$_6$.

For assembly **4a**$_3$·(DEB)$_{12}$, a good linear correlation between $\Delta\delta$NH^a and σ_p/σ_m was also observed for phenols **5a**, **5b**, **5f**, **5g**, **5h** and **5i** (bearing *p*-NO_2, *m*-NO_2, *p*-COOMe, *p*-Br, *p*-F, and H substituents, respectively). Again the observed shift was larger when using **5j** (bearing *p*-OMe) than expected based on the Hammett parameter σ_p.

The Hammett plots in Fig. 6 clearly show that the acidity of the hydroxy group is of great importance for phenol complexation by receptors **3b**$_3$·(DEB)$_6$ and **4a**$_3$·(DEB)$_{12}$. This importance is further emphasized by the other phenol derivatives. The addition of 10 equiv. of phenol (**5i**) (pK_a = 9.89) showed a shift of 0.06 ppm for H^m in the ^{1}H NMR spectrum. The addition of other less acidic phenols with pK_a values around 10, such as *p*-cresol (**5e**) and 4-fluorophenol (**5h**), showed much smaller shifts for H^m. Addition of 4-cyanophenol (**5c**), with a pK_a of 7.97, showed a large downfield shift for H^m ($\Delta\delta$ 0.22 ppm, 10 equiv. **5c**), comparable to the H^m shift caused by addition of *p*-nitrophenol **5a** (10 equiv., pK_a = 7.15). Determination of the binding constant of **5c** to assembly **3b**$_3$·(DEB)$_6$ by a ^{1}H NMR titration (1 mM, $CDCl_3$, 298 K) yielded an intrinsic K_a of 195 M^{-1} for formation of the 1 : 6 complex, a value that is on the same order as the intinsic K_a obtained for **5a** (202 M^{-1}).

We also studied the complexation of guest molecules containing two hydroxy groups. The addition of catechol (**5m**, 9–10 equiv.), which has a pK_a of 9.85, showed a downfield shift of the urea NH signal H^m of 0.19 ppm. The obtained shift of H^m is larger than expected based on the pK_a value of this phenol guest. However, pK_a values are generally measured in aqueous solutions, where the intramolecular bond between the two hydroxy groups does not occur. In apolar solutions, such as chloroform, this intramolecular hydrogen bond occurs and therefore the proton that is not involved in the hydrogen bond becomes more acidic. Catechol (**5m**) is unique in that it has two hydroxy groups *ortho* to each other. Therefore in principle two hydrogen bonds to the carbonyl of the ureido functionality can be formed by **5m**. If two hydrogen bonds are formed one would expect the complex to be stronger. Because of the low solubility of **5m** in $CDCl_3$, the intrinsic K_a could not be determined by ^{1}H NMR titration. If the two hydroxy functionalities are further

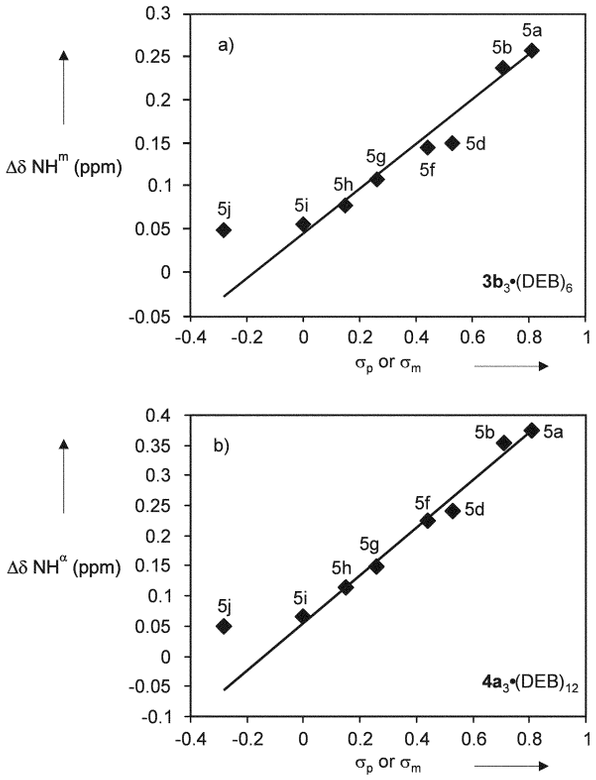


Fig. 6 Hammett plots for the NMR signal of NH$^{m/a}$ in the assemblies (a) $\mathbf{3b}_3$·(DEB)$_6$ and (b) $\mathbf{4a}_3$·(DEB)$_{12}$ (1 mM) in the presence of **5a**–**b**, **5d**, and **5f**–**j** (10 equiv.) in $CDCl_3$ at 293 K.

apart, as in resorcinol (**5l**, pK_a = 9.81), the observed shift of H^m after the addition of **5l** is much smaller ($\Delta\delta$ 0.07 ppm, 8 equiv.). The formation of the second hydrogen bond is probably not possible because of the geometry of the complex. Also the intramolecular hydrogen bond acidifying the other proton cannot occur.

Upon addition of **7** (bearing 2 OH-groups, 10 equiv.) to assembly $\mathbf{3b}_3$·(DEB)$_6$, a 0.16 ppm downfield shift was observed for NH^m. The observed shift is larger than expected based on the acidity of **7** (pK_a estimated around 10). The two hydroxy functionalities are too far apart to form either an intramolecular hydrogen bond or two hydrogen bonds with the same carbonyl functionality as for **5m**, therefore we suggest a two-point interaction (with two different carbonyl functionalities). A similar result (0.16 ppm downfield shift of NH^a) was also obtained upon addition of 10 equiv. of this guest to the *endo*-receptor $\mathbf{4a}_3$·(DEB)$_{12}$.

Conclusions

Complexation of phenol derivatives **5** is achieved by using both double- and tetrarosettes. For the *exo*-receptors $\mathbf{3b}_3$·(DEB)$_6$/(CYA)$_6$ and $\mathbf{3d}_3$·(DEB)$_6$ a 1 : 6 binding stoichiometry is observed. The guest molecules are complexed on both the top and bottom of the assembly. In contrast $\mathbf{4}_3$·(DEB)$_{12}$/(CYA)$_{12}$ are *endo*-receptors that show a 1 : 4 binding stoichiometry as determined by Job plot and MALDI-TOF MS. The complexation of **5a** inside the cavity between the second and third floors of the tetrarosette is mainly supported by VT NMR measurements. Splitting of the ^{1}H NMR signals for the NH$_{DEB}$-protons was observed at low temperatures upon addition of **5a**, indicating slow exchange between the free host $\mathbf{4}_3$·(DEB)$_{12}$ and different complexed hosts $\mathbf{4}_3$·(DEB)$_{12}$·$\mathbf{5a}_n$ (n = 1–4).

The pK_a of the phenol derivatives is important for the complexation with the ureido carbonyls in the double and tetrarosettes. Acidic phenol derivatives with pK_a values between 7.0–8.0 are the best binders. Phenol acidity, however, is not the only factor that influences binding to the rosette assemblies. Structural constraints in the guest can also be important. Thus, catechol a relatively poor acid with a pK_a of 9.85 is a good binder to $\mathbf{3b}_3$·(DEB)$_6$, probably due to the formation of two-point hydrogen bonds to the ureido group or to an intramolecular hydrogen bond. Addition of **7** clearly shows that formation of two hydrogen bonds strengthen the binding. The pK_a of the hydrogen bond accepting atom is also important for the binding. Accepting atoms with a low electron density are not capable of a strong complexation of **5a**. Furthermore, it has been shown that the double rosette contains six independent and well-organized binding sites and it has good potential as a noncovalent assembly for multivalent molecular recognition.

Acknowledgements

We thank Dr R. Fokkens for the MALDI-TOF measurements, Ing. T.W. Stevens for the FAB-MS measurements, Ms J. M. Visser for the VT NMR measurements, and The Technology Foundation for financial support (project number TST 4624, JK).

References

1 J. Ohkanda, J. W. Lockman, M. A. Kothare, Y. Qian, M. Blaskovich, S. Sebti and A. D. Hamilton, *J. Med. Chem.*, 2002, **45**, 177–188.
2 Recent review for synthetic receptors: J. H. Hartley, T. D. James and C. J. Ward, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3155–3184.
3 (*a*) D. J. Cram, T. Kaneda, R. C. Helgeson, B. Brown, C. B. Knobler, E. Maverick and K. N. Trueblood, *J. Am. Chem. Soc.*, 1985, **107**, 3645–3657; (*b*) T. W. Bell, A. B. Khasanov, M. G. B. Drew, A. Filikov and T. L. James, *Angew. Chem.*, 1999, **111**, 2705–2709 (*Angew. Chem., Int. Ed.*, 1999, **38**, 2543–2547); (*c*) L. A. J. Chrisstoffels, F. de Jong, D. N. Reinhoudt, S. Sivelli, L. Gazzola, A. Casnati and R. Ungaro, *J. Am. Chem. Soc.*, 1999, **121**, 10142–10151.
4 (*a*) P. A. Gale, *Coord. Chem. Rev.*, 2001, **213**, 79–128; (*b*) H. Miyaji, P. Anzenbacher Jr., J. L. Sessler, E. R. Bleasdale and P. A. Gale, *Chem. Commun.*, 1999, 1723–1724; (*c*) F. Sansone, L. Baldini, A. Casnati, M. Lazzarotto, F. Ugozzoli and R. Ungaro, *P. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 4842–4847; (*d*) M. Yamamoto, A. Sugasaki, M. Ikeda, M. Takeuchi, K. Frimat, T. D. James and S. Shinkai, *Chem. Lett.*, 2001, 520–521; (*e*) B. H. M. Snellink-Ruël, M. M. G. Antonisse, J. F. J. Engbersen, P. Timmerman and D. N. Reinhoudt, *Eur. J. Org. Chem.*, 2000, 165–170; (*f*) T. S. Snowden, A. P. Bisson and E. V. Anslyn, *J. Am. Chem. Soc.*, 1999, **121**, 6324–6325.
5 (*a*) O. Middel, W. Verboom and D. N. Reinhoudt, *Eur. J. Org. Chem.*, 2002, 2587–2597; (*b*) A. S. Droz and F. Diederich, *J. Chem. Soc., Perkin Trans. 1*, 2000, 4224–4226; (*c*) S. Tamaru, S. Shinkai, A. B. Khasanov and T. W. Bell, *Proc. Natl. Acad. Sci, U. S. A.*, 2002, **99**, 4972–4976; (*d*) S. D. Starnes, D. M. Rudkevich and J. Rebek, Jr, *J. Am. Chem. Soc.*, 2001, **123**, 4659–4669; (*e*) B. P. Orner, X. Salvatella, J. S. Quesada, J. de Mendoza, E. Giralt and A. D. Hamilton, *Angew. Chem.*, 2002, **114**, 125–127 (*Angew. Chem., Int. Ed.*, 2002, **41**, 117–119); (*f*) M. Almaraz, C. Raposo, M. Martín, M. C. Caballero and J. R. Morán, *J. Am. Chem. Soc.*, 1998, **120**, 3516–3517.
6 (*a*) M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem.*, 1998, **110**, 2908–2953 (*Angew. Chem., Int. Ed.*, 1998, **37**, 2754–2794); (*b*) Y. C. Lee and R. T. Lee, *Acc. Chem. Res.*, 1995, **28**, 321–327; (*c*) T. K. Lindhorst, *Top. Curr. Chem.*, 2002, **218**, 201–235.
7 E. Benjamini, G. Sunshine, S. Leskowitz in *Immunology, A Short Course*, 3rd ed. Wiley, New York, 1996.
8 (*a*) D. S. Goodsell and A. J. Olson, *Annu. Rev. Biophys. Biomol. Struct.*, 2000, **29**, 105–153; (*b*) Z. Zhang, E. A. Merritt, M. Ahn, C. Roach, Z. Hou, C. L. M. J. Verlinde, W. G. J. Hol and E. Fan, *J. Am. Chem. Soc.*, 2002, **124**, 12991–12998.
9 (*a*) D. W. P. M. Löwik, M. D. Weingarten, M. Broekema, A. J. Brouwer, W. C. Still and R. M. J. Liskamp, *Angew. Chem.*, 1998, **110**, 1947–1950 (*Angew. Chem., Int. Ed.*, 1998, **37**, 1846–1850) . Reviews about diversity generation; (*b*) Y. R. de Miguel and J. K. M. Sanders, *Curr. Opin. Chem. Biol.*, 1998, **2**, 417–421; (*c*) W. C. Still, *Acc. Chem. Res.*, 1996, **29**, 155–163; (*d*) J. J. Lavigne and E. V. Anslyn, *Angew. Chem.*, 2001, **113**, 3212–3225 (*Angew. Chem., Int. Ed.*, 2001, **40**, 3118–3130) . Examples of the introduction of multiple binding sites; (*e*) J. H. Rao, J. Lahiri, L. Isaacs, R. M. Weis and G. M. Whitesides, *Science*, 1998, **280**, 708–711; (*f*)

B. Colonna, V. D. Harding, S. A. Nepogodiev, F. M. Raymo, N. Spencer and J. F. Stoddart, *Chem. Eur. J.*, 1998, **4**, 1244–1254; (*g*) T. K. Lindhorst, *Top. Curr. Chem.*, 2002, **218**, 201–235; (*h*) A. M. A. van Wageningen and R. M. J. Liskamp, *Tetrahedron Lett.*, 1999, **40**, 9347–9351; (*i*) P. H. Rasmussen and J. Rebek Jr., *Tetrahedron Lett.*, 1999, **40**, 3511–3514; (*j*) I. Vrasidas, N. J. de Mol, R. M. J. Liskamp and R. J. Pieters, *Eur. J. Org. Chem.*, 2001, 4685–4692; (*k*) B. G. Davis, *J. Chem. Soc., Perkin Trans. 1*, 1999, 3215–3237; (*l*) N. Jayaraman, S. A. Nepogodiev and J. F. Stoddart, *Chem. Eur. J.*, 1997, **3**, 1193–1199; (*m*) A. Dondoni, M. Kleban, X. Hu, A. Marra and H. D. Banks, *J. Org. Chem.*, 2002, **67**, 4722–4733.

10 (*a*) R. Boyce, G. Li, H. P. Nestler, T. Suenaga and W. C. Still, *J. Am. Chem. Soc.*, 1994, **116**, 7955–7956; (*b*) Y. Cheng, T. Suenaga and W. C. Still, *J. Am. Chem. Soc.*, 1996, **118**, 1813–1814.

11 R. Fiammengo, M. Crego-Calama, P. Timmerman and D. N. Reinhoudt, *Chem. Eur. J.*, 2003, **9**, 784–792.

12 Y. Hamuro, M. Crego-Calama, H. S. Park and A. D. Hamilton, *Angew. Chem.*, 1997, **109**, 2797–2800 (*Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2680–2683).

13 (*a*) M. Crego-Calama, R. Hulst, R. Fokkens, N. M. M. Nibbering, P. Timmerman and D. N. Reinhoudt, *Chem. Commun.*, 1998, 1021–1022; (*b*) M. Crego-Calama, P. Timmerman and D. N. Reinhoudt, *Angew. Chem.*, 2000, **112**, 771–774 (*Angew. Chem., Int. Ed.*, 2000, **39**, 755–758).

14 (*a*) O. Ramström and J.-M. Lehn, *Nature Reviews, Drug Discovery*, 2002, **1**, 26–36; (*b*) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders and J. F. Stoddart, *Angew. Chem.*, 2002, **114**, 938–993 (*Angew. Chem., Int. Ed.*, 2002, **41**, 898–952).

15 (*a*) M. S. Goodman, V. Jubian, B. Linton and A. D. Hamilton, *J. Am. Chem. Soc.*, 1995, **117**, 11610–11611; (*b*) G. R. L. Cousins, R. L. E. Furlan, Y.-F. Ng, J. E. Redman and J. K. M. Sanders, *Angew. Chem.*, 2001, **113**, 437–442 (*Angew. Chem., Int. Ed.*, 2001, **40**, 423–428); (*c*) G. R. L. Cousins, S.-A. Poulsen and J. K. M. Sanders, *Chem. Commun.*, 1999, 1575–1576. Examples of diversity generation and amplification can be found in; (*d*) V. Goral, M. N. Nelen, A. V. Eliseev and J.-M. Lehn, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 1347–1352; (*e*) A. V. Eliseev and M. N. Nelen, *J. Am. Chem. Soc.*, 1997, **119**, 1147–1148; (*f*) R. L. E. Furlan, Y.-F. Ng, S. Otto and J. M. K. Sanders, *J. Am. Chem. Soc.*, 2001, **123**, 8876–8877; (*g*) I. Huc and J.-M. Lehn, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 2106–2110.

16 (*a*) R. H. Vreekamp, J. P. M. van Duynhoven, M. Hubert, W. Verboom and D. N. Reinhoudt, *Angew. Chem.*, 1996, **108**, 1306–1309 (*Angew. Chem., Int. Ed.*, 1996, **35**, 1215–1218); (*b*) P. Timmerman, R. H. Vreekamp, R. Hulst, W. Verboom, D. N. Reinhoudt, K. Rissanen, K. A. Udachin and J. Ripmeester, *Chem. Eur. J.*, 1997, **3**, 1823–1832.

17 (*a*) K. A. Jolliffe, P. Timmerman and D. N. Reinhoudt, *Angew. Chem.*, 1999, **111**, 983–986 (*Angew. Chem., Int. Ed.*, 1999, **38**, 933–937); (*b*) L. J. Prins, E. E. Neuteboom, V. Parashiv, M. Crego-Calama, P. Timmerman and D. N. Reinhoudt, *J. Org. Chem.*, 2002, **67**, 4808–4820.

18 J. M. C. A. Kerckhoffs, M. Crego-Calama, I. Luyten, P. Timmerman and D. N. Reinhoudt, *Org. Lett.*, 2000, **2**, 4121–4124.

19 (*a*) K. Matsuura, M. Hibino, Y. Yamada and K. Kobayashi, *J. Am. Chem. Soc.*, 2001, **123**, 357–358; (*b*) S. Sakai and T. Sasaki, *J. Am. Chem. Soc.*, 1994, **116**, 1587–1588.

20 (*a*) M. Herm, O. Molt and T. Schrader, *Chem. Eur. J.*, 2002, **8**, 1485–1499; (*b*) A. R. Bernardo, J. F. Stoddard and A. E. Kaifer, *J. Am. Chem. Soc.*, 1992, **114**, 10624–10613.

21 J. B. Harborne and C. A. Williams, *Phytochemistry*, 2000, **55**, 481–504.

22 M. D. Krasowski, X. Hong, A. J. Hopfinger and N. L. Harrison, *J. Med. Chem.*, 2002, **45**, 3210–3221.

23 For a small selection of covalent synthetic receptors for phenolic guests see: (*a*) R. J. Jansen, R. de Gelder, A. E. Rowan, H. W. Scheeren and R. J. M. Nolte, *J. Org. Chem.*, 2001, **66**, 2643–2653; (*b*) B. J. Whitlock and H. W. Whitlock, *J. Am. Chem. Soc.*, 1994, **116**(6), 2301–2311; (*c*) J. E. Cochran, T. J. Parrott, B. J. Whitlock and H. W. Whitlock, *J. Am. Chem. Soc.*, 1992, **114**(6), 2269–2270; (*d*) J. N. H. Reek, A. H. Priem, H. Engelkamp, A. E. Rowan, J. A. A. W. Elemans and R. J. M. Nolte, *J. Am. Chem. Soc.*, 1997, **119**(42), 9956–9964.

24 The program Microsoft Excel and a nonlinear least-square fitting procedure is used.

25 Two different K_a's of 25 and 180 M^{-1} for the formation of two hydrogen bonds between resorcinol and two urea carbonyl goups were found (see ref 23); The obtained K_a depends also on the substituent on the phenol.

26 (*a*) P. Timmerman, K. A. Jolliffe, M. Crego-Calama, J.-L. Weidmann, L. J. Prins, F. Cardullo, B. H. M. Snellink-Ruel, R. Fokkens, N. M. M. Nibbering, S. Shinkai and D. N. Reinhoudt, *Chem. Eur. J.*, 2000, **6**, 4104–4115; (*b*) D. W. Amstrong, L.-K. Zhang, L. He and M. L. Gross, *Anal. Chem.*, 2001, **73**, 3679–3686 and their references therein; (*c*) S. Ring and Y. Rudich, *Rapid Commun. Mass Spectrom.*, 2000, **14**, 515–519; (*d*) T. Fujita, Y. Itagaki, M. Hisaka, H. Naoki, T. Nakajima and M. Andriantsiferana, *Rapid Commun. Mass Spectrom.*, 1997, **11**, 1115–1119.

27 Besides large intensity signals, corresponding to the protonated and Ag^+-cationized molecules of the building blocks (at *m/z* 1181 and *m/z* 1288 respectively), clusters of ions (intensities of TIC (Total Ion Current) between <1% and 12%) could be observed in the high mass range. Among unidentified signals in the mass range 3000–7000, the signals for the complexes of $\mathbf{3b_3}\cdot(DEB)_6$ with **5a** were detected, which were all in agreement with their theoretical mass assignment, according to their isotopic pattern. In all experiments no quantitative observations could be made due to ion fluctuations of the clusters observed in the high mass range.

28 Broadening of protons $H^{a/b}$ (NH protons of DEB) is also observed in assembly $\mathbf{2_3}\cdot(DEB)_6$. This indicates that this proton is in fast exchange. This observed broadening is also an indication of the lower stability of $\mathbf{2_3}\cdot(DEB)_6$.

29 pK_a of *N,N′*-dibutylmelamine is 5.78 [34a] and pK_a of **5a** is 7.15.

30 (*a*) B. A. Murray and G. S. Whelan, *Pure Appl. Chem.*, 1996, **68**, 1561–1567; (*b*) G. T. W. Gieling, H. W. Scheeren, R. Israël and R. J. M. Nolte, *Chem. Commun.*, 1996, 241–244.

31 Gas-phase molecular modeling calculations (Quanta 97, CHARMm 24.0) of the empty assembly showed that the cavity between the second and third floors is completely shielded from the environment and that the six ureido carbonyl groups are pointing to the center of this endohedral cavity.

32 In a control system, the assembly $\mathbf{8_3}\cdot(DEB)_{12}$, where tetramelamine **8** does not bear ureido moieties (for drawing of compound **8** see: compound **2b** in ref 17*a*), the broadening of the NH^{a1} and NH^{b1} peaks occurred along with no shifts upon the addition of **5a** ($\delta_{1/2}$, from 5.6 Hz to 26 Hz for NH^{a1}, 10 equiv. **5a**). On the other hand, such a broadening does not occur for the second and third floors ($\delta_{1/2}$, 5.4 Hz for NH^{a2}, 0 and 10 equiv. **5a**). The results suggest that the proton exchange occurs between the OH proton of **5a** and the NH^{a1}/NH^{b1} protons of the first and fourth floors. For $\mathbf{4b_3}\cdot(DEB)_{12}$, the peak broadening ($\delta_{1/2}$, from 8.4 Hz to 26 Hz for NH^{a1}, 10 equiv. **5a**) was more significant compared to that of the achiral $\mathbf{4a_3}\cdot(DEB)_{12}$ system ($\delta_{1/2}$, from 6.0 Hz to 8.4 Hz for NH^{a1}, 10 equiv. of **5a**), indicating the facilitation of the proton exchange in the chiral system. For $\mathbf{4b_3}\cdot(BuCYA)_{12}$ the peak broadening of the NH^{a1} and NH^{b1} was hardly observed ($\delta_{1/2}$, from 6.4 Hz to 6.7 Hz for NH^{a1}, 10 equiv. **5a**).

33 In the absence of **5a**, $\mathbf{4b_3}\cdot(DEB)_{12}$ is strongly CD active due to the assymmetric arrangement of the many different chromophoric units within the assembly (L. J. Prins, J. Huskens, F. de Jong, P. Timmerman and D. N. Reinhoudt, *Nature*, 1999, **398**, 498–502) The strong CD band around 290 nm decreased upon the addition of **5a**, indicating the decomposition of the tetrarosette assembly initiated by the significant proton exchange. The CD intensity was reduced to *ca.* 40% (20 equiv. **5a**), which is comparable to the assembly formation of 40% estimated from the ^{1}H NMR method. On the other hand, for $\mathbf{4b_3}\cdot(BuCYA)_{12}$, the CD intensity around 290 nm hardly decreased upon the addition of **5a**. This phenomenon is ascribed to the high stability of $\mathbf{4b_3}\cdot(BuCYA)_{12}$ as suggested by the ^{1}H NMR study.

34 (*a*) M. Mascal, P. S. Fallon, A. S. Batsanov, B. R. Heywood, S. Champ and M. Colclough, *J. Chem. Soc., Chem. Commun.*, 1995, 805–806; (*b*) A. Bielejewska, C. Marjo, L. J. Prins, P. Timmerman, F. de Jong and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2001, **123**, 7518–7533.

35 K. A. Connors, in *Binding Constants*, Wiley, New York, 1987.

36 However, the large K_a value of 299 $mol^{-1}dm^3$ in the $\mathbf{4b_3}\cdot(DEB)_{12}$ system is not very reliable because $\mathbf{4b_3}\cdot(DEB)_{12}$ dissociated upon the addition of more than 10 equiv. **5a**.

37 J. March, in *Advanced Organic Chemistry*, 4th ed., Wiley, New York, 1992, p. 278–286; . Changes in chemical shift do not necessarily reflect the strength of the complex being formed. Nevertheless, assuming that all the complexes are formed in a similar way, we use changes in chemical shift as a rough estimation of the complex strength.

38 P. R. Ashton, M. C. T. Fyfe, S. K. Hickingbottom, J. F. Stoddard, A. J. P. White and D. J. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2117–2128.