Anion Complexation by Glycocluster Thioureamethyl Cavitands: Novel ESI-MS-Based Methods for the Determination of K_a Values

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Abstract: A series of saccharide-thiourea functionalized cavitands was prepared in good yields (72–86%) by reaction of a tetrakis(aminomethyl) cavitand with the thiocyanate derivatives of acetylated glucose, galactose, and cellobiose. The anion complexation behavior of the acetylated and deacetylated glycocluster thioureamethyl cavitands was studied with electrospray ionization mass spectrometry (ESI-MS) in acetonitrile and in a 1:1 acetonitrile/water mixture, respectively. All compounds show a preference for Cl^-. A linear relationship was found between the square root of the intensity and the concentration of the formed host–guest complex. Based on this relationship, novel methods have been developed to determine K_a values, by means of direct titration and competition experiments.

Keywords: anions · cavitands · mass spectrometry · molecular recognition · receptors

Introduction

Molecular recognition is the most important phenomenon in supramolecular chemistry.[1] A number of established analytical methods are being used to study the strength of the host–guest interactions. The most commonly used methods include NMR and UV spectroscopy, calorimetry, and electrochemical techniques.[2]

Electrospray ionization mass spectrometry (ESI-MS) has been applied to directly determine binding constants of large biological complexes.[3] From titration experiments, the intensities of both the free and the bound guest (or the free and bound host) are used to derive a Scatchard plot. The prerequisite is that the host or the guest and the host–guest complex are ionic; however, this approach is not applicable when the guest is a small ion. Recently, ESI-MS has been used to determine the binding constants of crown-ether alkali-metal cation complexes by means of competition experiments.[4] Very recently, Russell et al. reported a qualitative study of the anion complexation by polydentate Lewis acids by the use of nanoelectrospray MS.[5] However, to the best of our knowledge, ESI-MS has not been used to study anion complexation *quantitatively.*

Resorcinarene-based cavitands with proper ligating sites give rise to different types of receptors.[6] Previously, we have demonstrated that thiourea functionalized cavitands are good receptors for halides in chloroform with a preference for chloride.[7] With the ultimate objective of mimicking nature, the study of supramolecular interactions in aqueous media is very challenging. Several groups have reported the synthesis of cavitands with an enhanced solubility in water. This requires the introduction of either charged moieties, such as quaternary ammonium,[8] amidinium,[9] pyridinium,[10] carboxylate,[11] phosphoric acid salt,[12] phenoxide,[13] etc., or neutral substituents, such as peptides,[14] diethanolamine,[15] dendritic wedges,[16] Pd-organometallic centers.[17] Saccharides have been used as water-solubilizing moieties in the cases of calix[4]arenes[18] and resorcinols,[19] and there is one example of a cavitand.[20]

Herein we report the synthesis of cavitands containing both saccharide[21] and thiourea moieties and their complexation with anions[22] in polar solvents. We describe novel methods for the determination of the binding constants of the anion complexation by means of ESI-MS.

Results and Discussion

Synthesis: The synthesis of the saccharide-thiourea functionalized cavitands **11–14** is summarized in Scheme 1.

Tetrakis(aminomethyl) cavitand **5** was prepared starting from the corresponding bromomethyl cavitand[23] **1** by reaction with potassium phthalimide followed by deprotection of phthalimido cavitand **2** with hydrazine hydrate, analogously

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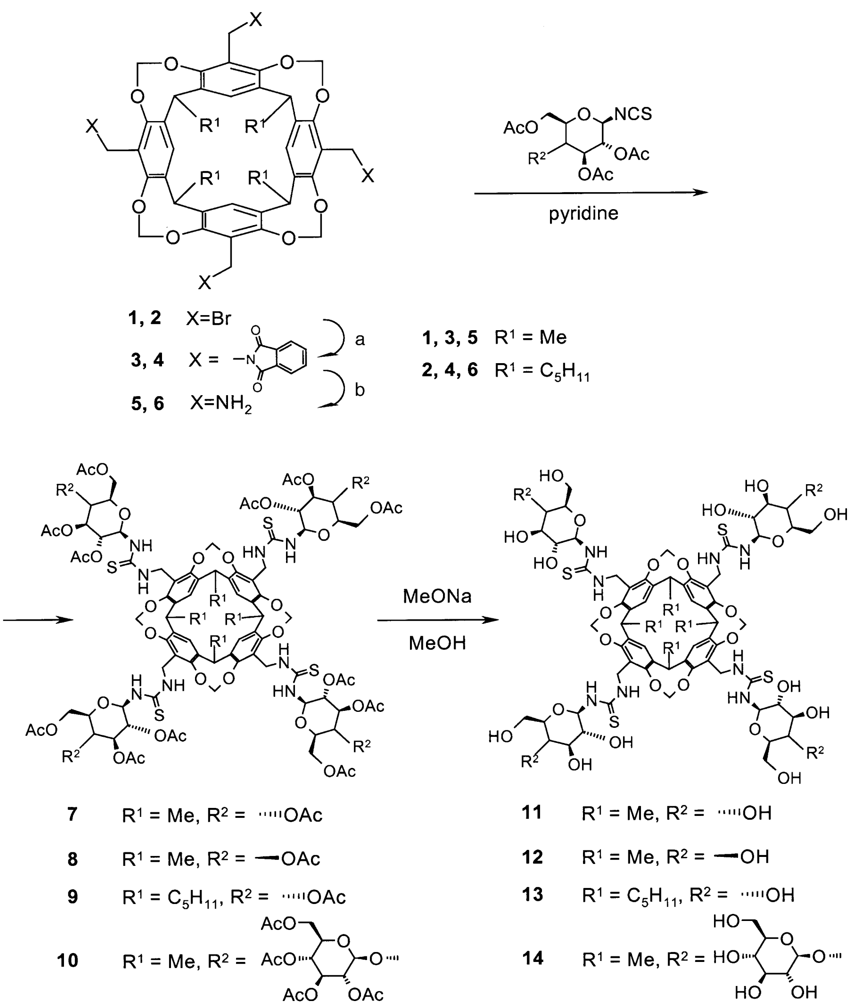


1, 2 X=Br

3, 4 X = phthalimido

5, 6 X=NH_2

1, 3, 5 R^1 = Me

2, 4, 6 R^1 = C_5H_{11}

7 R^1 = Me, R^2 = OAc

8 R^1 = Me, R^2 = OAc

9 R^1 = C_5H_{11}, R^2 = OAc

10 R^1 = Me, R^2 =

11 R^1 = Me, R^2 = OH

12 R^1 = Me, R^2 = OH

13 R^1 = C_5H_{11}, R^2 = OH

14 R^1 = Me, R^2 =

Scheme 1. Synthesis of glycocluster thioureamethyl cavitands. a) Potassium phthalimide, MeCN. b) Hydrazine hydrate, MeOH).

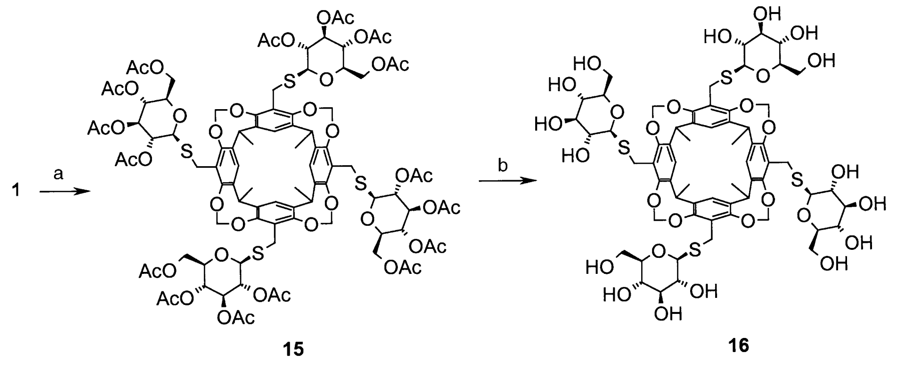
to the synthesis of the previously reported pentyl derivative **6**.

Reaction of tetrakis(aminomethyl) cavitand **5** with the thiocyanate derivatives of acetylated glucose, galactose, and cellobiose in pyridine at room temperature afforded the acetyl-protected saccharide-thiourea functionalized cavitands **7**, **8**, and **10** in 80, 85, and 86 % yield, respectively. In an analogous manner, cavitand **9** was prepared in 72 % yield by reaction of pentyl derivative **6** with acetylated glucosyl isothiocyanate. Subsequent deacetylation of compounds **7**, **8**, **9**, and **10** with sodium methoxide in a 4:1 mixture of methanol and dichloromethane gave saccharide-thiourea functionalized cavitands **11–14** in 96, 95, 90, and 92 % yield, respectively.

To study the influence of the thiourea linker between the cavitand and the saccharide moieties, cavitand **16** with SCH_2 linkers was prepared (Scheme 2). Reaction of tetrakis(bromomethyl) cavitand **1** with 2,3,4,5-tetra-*O*-acetyl-β-D-glucopyranosylthiol in dichloromethane gave gluco-thiamethyl cavitand **15** in 88 % yield. Subsequent deacetylation with a catalytic amount of sodium methoxide afforded cavitand **16** in 91 % yield.

The formation of both the acetylated (**7**–**10**) and deacetylated cavitands (**11**–**14**) clearly followed from the MALDI mass spectra and satisfactory elemental analyses. Their ^1H NMR spectra in $CDCl_3$ and CD_3CN only exhibit broad signals. However, spectra with sharp signals were obtained in $[D_6]DMSO$ solutions. Characteristic is the signal at $\delta = 5.8$ and 5.2 ppm of the anomeric CH of the sugars in the acetylated and deacetylated compounds, respectively.

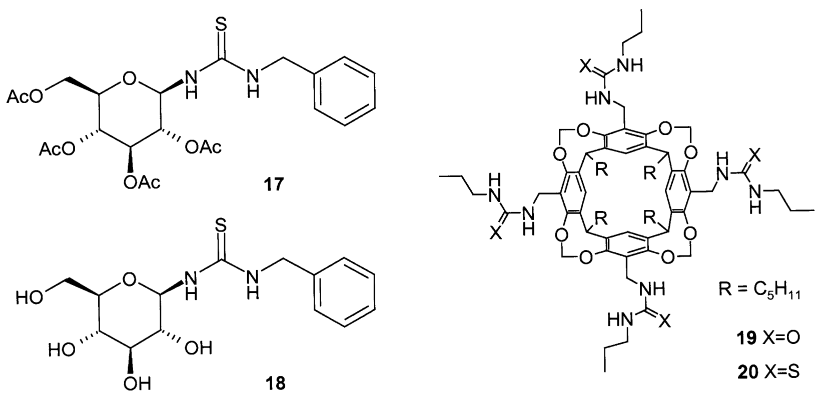
In acetonitrile, the solubility of the acetylated glycocluster thiourea cavitands **7**–**10** is twice as high as that of the propylthiourea cavitand **20** ($\approx$5 versus 2.2 $\mathrm{mM\,L^{-1}}$). The solubility of the corresponding propylurea cavitand **19** in acetonitrile is 0.4 $\mathrm{mM\,L^{-1}}$. The solubilities of glycocluster thiourea cavitands **11–14** in water are 0.5, 0.6, 0.8, and $>$300 $\mathrm{mM\,L^{-1}}$, respectively, while that of compound **15** is 1 $\mathrm{mM\,L^{-1}}$. The much higher solubility of cavitand **14** in water clearly shows the influence of four disaccharide moieties. The solubility of **14** in water is even higher than that of a cavitand containing dendritic wedges with a total of 45 tetraethylene glycol chains (221 $\mathrm{mM\,L^{-1}}$).[16]



15

16

Scheme 2. Synthesis of cavitand glycoclusters containing sulfide linkers. a) 2,3,4,6-Tetraacetyl-1-thiaglucose, Na_2CO_3. b) MeONa, MeOH, CH_2Cl_2.



Scheme 3. Reference compounds.

Simpler analogues of cavitand glycoclusters, used for competitive experiments, are shown in Scheme 3.

Anion complexation behavior studied by ESI-MS:

General: The anion complexing ability of both the acetylated (**7**–**10**, **15**) and deacylated glycocluster thiourea cavitands **11**–**14**, **16** clearly followed from the signal of the 1:1 complexes in the ESI-MS negative ion mode spectra. Since in the ^{1}H NMR spectra rather broad signals were obtained, ^{1}H NMR spectroscopy was not suitable to determine K_a values of the complexes. Therefore we investigated whether ESI-MS can be used to determine K_a values.

In ESI-MS, charged host–guest complexes are transferred from small droplets of solution into the gas phase under relatively mild conditions.[24] In general, this does not give rise to fragmentation. Some parameters involve: 1) properties of the species measured, for example, ionization potential and volatility, 2) the properties of the solvent used, for example, conductivity and surface tension, 3) the co-solutes present, and 4) the settings of the mass spectrometer, such as applied voltage, temperature, gas flow, etc. However, all these parameters remain constant during a set of experiments. We investigated whether there is a relationship between the intensity of the signal of the 1:1 complex and the concentration of the complex in the solution.

As a model experiment, the chloride complexation of acetylated glucose functionalized thiourea cavitand **7** was studied in MeCN. Two different kinds of titrations were carried out: titration of Bu_4NCl with **7** (keeping the total concentration of the chloride constant), and titration of host **7** with Bu_4NCl (with a constant total host concentration). For both titrations the relationship between the intensity of the signal of the host–guest complex **7**@Cl^- and its concentration is depicted in Figure 1a and 1b, respectively. In Figure 1a, the peak intensity increases parabolically, which is very clear compared to Figure 1c, which depicts the square root of the peak intensity vs the concentration of the host–guest complex.[25] The relationship fits with Equation (1), where *I* is the peak intensity and A is a constant that includes the properties of the complex, the settings of the mass spectrometer, etc. (vide supra).

$$I = \mathrm{A} \times [HG]^2 \tag{1}$$

In the case of Figure 1b, the increase of the peak intensity of the host–guest complex is followed by a decrease. This so-called suppression[24c] is caused by a co-solute (in our case the titrant) that is present in the measuring solution. A co-

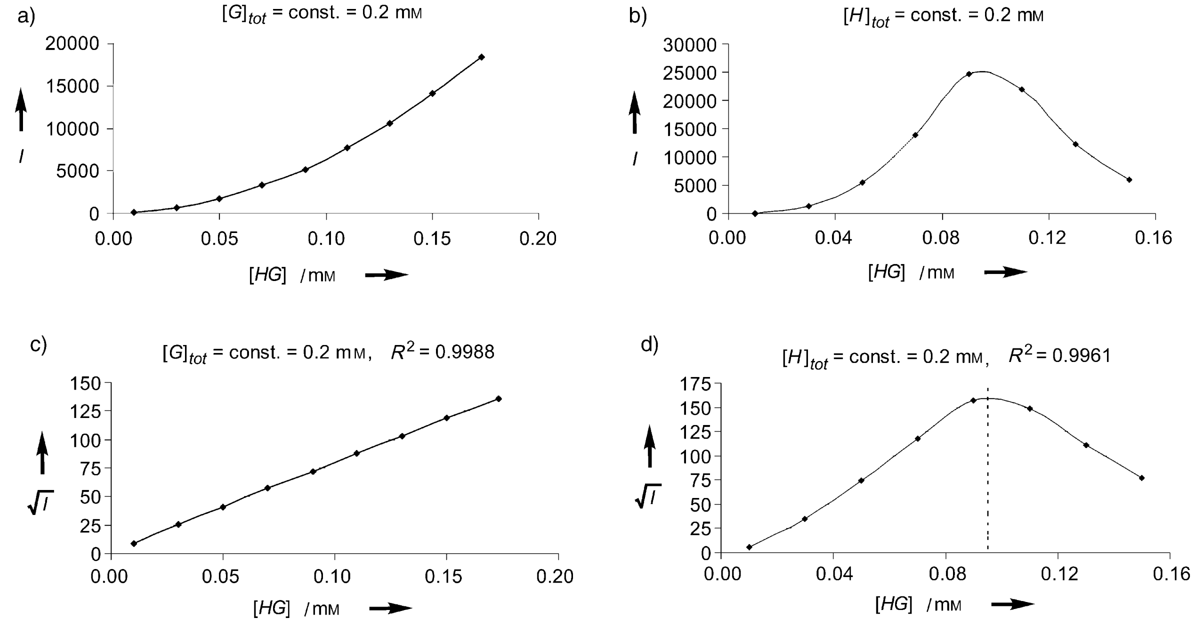


Figure 1. a) Titration of Bu_4NCl with **7**, intensity versus concentration of **7**@Cl^-. b) Titration of **7** with Bu_4NCl, intensity versus concentration of **7**@Cl^-. c) Titration of Bu_4NCl with **7**, $\sqrt{}$intensity versus concentration of **7**@Cl^-. d) Titration of **7** with Bu_4NCl, $\sqrt{}$intensity versus concentration of **7**@Cl^-.

solute can prevent the formation of droplets of the required small size as well as decrease the volatization efficiency of the measured ions from the formed droplets, since they occupy its surface. In general, electrolytes cause a higher suppression than non-electrolytes. Figure 1 d shows that the same quadratic relationship as Equation (1) is obeyed until suppression starts to play a role.

Direct method for K_a value determination by ESI-MS titration: The relationship found [Eq. (1)] can be used to determine association constants. When the concentration of the anion ([G]) is maintained constant, the concentration of the host–guest complex ([HG]) can be calculated at each moment from the intensity I of the signal of the complex with Equation (2).

$$[HG] = [HG]_{\max}\frac{\sqrt{I}}{\sqrt{I_{\max}}} \qquad (2)$$

$$[HG] = [G]_{\mathrm{tot}}\frac{\sqrt{I}}{\sqrt{I_{\max}}} \qquad (3)$$

In Equation (2), $I_{\max}$ is the intensity when $[HG] = [HG]_{\max}$. Since the maximum concentration of the host–guest complex $[HG]_{\max}$ is equal to the total concentration of the anionic guest $[G]_{\mathrm{tot}}$, substitution gives a relationship between the intensity of the signal and the concentration of the host–guest complex [HG] and $[G]_{\mathrm{tot}}$ [Eq. (3)].

Nonlinear fitting of the experimental data I_{exp}, $[G]_{\mathrm{tot}}$, and $[H]_{\mathrm{tot}}$ with the calculated value of the intensity I_{calcd} by varying $\sqrt{I_{\max}}$ and K_a ultimately gives the calculated association constant K_a. An example of the calculated and the experimental ESI-MS data of a titration of acetylated galactose functionalized thiourea cavitand **8** with Bu_4NCl is presented in Figure 2.

This methodology was used to study the chloride complexation of acetylated glycocluster thiourea cavitands **7**–**9** in MeCN; the K_a values are summarized in Table 1. In the case of lower binding constants, the larger amount of host necessary to give a sufficient amount of host–guest complex may also give rise to suppression (vide supra) of the peak intensity. Nevertheless, the K_a values can be calculated by taking into account only the non-suppression area of the ESI-MS titration curve (compare Figure 1 d). In this way, a K_a value of 720 M^{-1} (with ITC $K_a = 680\,\mathrm{M}^{-1}$) was obtained for the complexation of compound **7** with Bu_4NBr in MeCN.

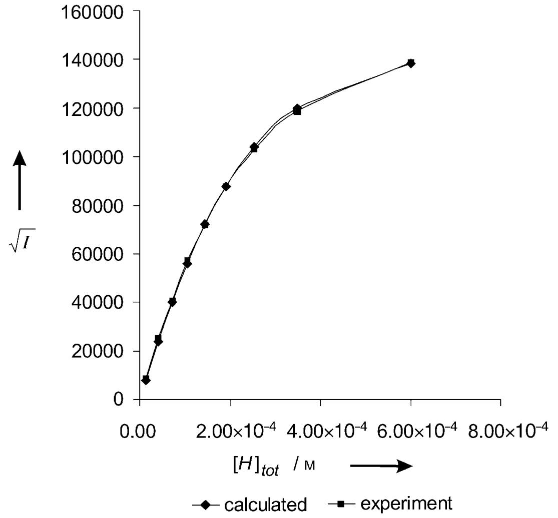


Figure 2. Nonlinear curve fitting of data of ESI-MS titration of **8** with Bu_4NCl. The titration data are within the Weber concentration range of 20–80 % complex formation.

Table 1. ESI-MS and ITC data of the complexation of Cl^- by compounds **7**–**9** in MeCN.

Compound	ESI-MS	Isothermal microcalorimetry data			
	K_a [M^{-1}]	K_a [M^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	ΔS [kcal mol^{-1}]
7	15100	15000	−5.694	−4.685	1.009
8	15000	14700	−5.685	−4.271	1.414
9	14500	14600	−5.657	−4.283	1.374

In all cases, the K_a values found are in excellent agreement with those obtained with isothermal microcalorimetry (ITC) (Table 1), confirming the validity of the described ESI-MS titration method.

Calibrated competitive method: Some years ago Kempen and Brodbelt[4a] reported a method for the determination of binding constants of crown ethers with cations by means of ESI-MS. The peak intensity of a reference host–guest complex with a known K_a value was monitored before and after addition of a second host or guest. The K_a value of the new complex is calculated on the basis of the change in intensity of the reference complex and extrapolation from a calibration curve ([HG] versus I_{HG}).[26] To obtain reliable results, the difference in the K_a values involved should be within two orders of magnitude.[27]

We have used this methodology to study anion complexation by addition of a new host to an existing host–guest complex. The determination of the K_a value of **19**@Cl^- was studied in more detail. A set of solutions with different concentrations of propylthiourea cavitand **19** in MeCN solution containing constant initial total concentration of acetylated glucose functionalized thiourea cavitand **7** and Bu_4NCl were prepared. From a calibration curve ($[H_1G]$ vs $\sqrt{I_{H_1G}}$) the changes in the intensities of **7**@Cl^- were transformed to its concentration and used for the calculation of the K_a value of **19**@Cl^- (Table 2). The averaged K_a value of entries 2–4 in

Table 2. K_a values of **19**@Cl^- with the ESI-MS calibrated competitive method with **7**@Cl^- as the reference complex.

Entry	Concentration[a] [mM]		K_a (**19**@Cl^-) [M^{-1}]
	19	[**7**@Cl^-]	
1	0	0.11	–
2	0.1	0.09	17200
3	0.2	0.074	17100
4	0.3	0.062	17000
5	0.4	0.047	22400

[a] Initial concentrations of Bu_4NCl and **7** were 0.2 mM and 0.191 mM, respectively.

Table 2 is $17100 \pm 100\,\text{M}^{-1}$, in agreement with the K_a value of $17300\,\text{M}^{-1}$ determined with ITC. However, the K_a value in entry 5 in Table 2 is much too high, originating from suppression caused by the higher competitor concentration. This also illustrates the disadvantage of this method. Several experiments must always be carried out with different competitor concentrations. When at least two of the initial K_a values were within the experimental error, much higher K_a values caused by suppression were excluded.

Complex **7**@Cl$^-$ was used as the reference to study the Cl$^-$ complexation of compounds **9**, **10**, **15**, **17**, and **20** in MeCN; the K_a values are summarized in Table 3. The choice of the reference complex has hardly any effect. Competition experiments of compounds **7**@Cl$^-$ or **8**@Cl$^-$ with **17** in MeCN gave K_a values of 450 and $440\,\text{M}^{-1}$, respectively. The K_a values are very close to that obtained with ITC, namely, $430\,\text{M}^{-1}$. These experiments show the possibility to determine relatively low K_a values using this methodology.

Table 3. K_a values of Cl$^-$ complexation by different hosts in MeCN obtained with the ESI-MS calibrated competitive method with **7**@Cl$^-$ as the reference complex.

Entry	Host	K_a [M^{-1}]
1	**9**	14900
2	**10**	4600
3	**15**	5200
4	**17**	450 (440[a], 430[b])
5	**20**	15400

[a] **8**@Cl$^-$ as the reference complex. [b] ITC data.

Competition experiments of the reference complexes **7**@Cl$^-$ and **8**@Cl$^-$ with Br$^-$, as an anionic guest, showed considerable suppression of the signal. Upon titration with Br$^-$, the intensity of the reference complex dropped much more than expected, even if only small amounts of Br$^-$ were added. Suppression was even observed upon addition of small amounts of the anions PF_6^- and BPh_4^-. Since anionic species cause significant suppression, this methodology cannot be used to study the complexation behavior of a host toward a variety of anions.

Competition method: On account of the above-mentioned problems, a novel, relatively simple competitive method was developed.[28] For this approach, the K_a value of one host–guest complex has to be known, determined by the direct ESI-MS titration (vide supra) or another method. Since we are dealing with rather large hosts and relatively small guests, we assumed that the volatility of the different complexes is (almost) equal. This means that Equation (4) can be derived from Equation (1). Equation (4) describes the ratio of the concentration of the two *HG* complexes as the ratio of the square roots of the respective intensities.

$$\frac{[HG_1]}{[HG_2]} = \frac{\sqrt{I_1}}{\sqrt{I_2}} \tag{4}$$

Together with the mass balance Equations (5)–(7) and the binding constants Equations (8) and (9), the different unknowns in the equations can be solved to give the K_{a2} value.

$$[HG_1] + [HG_2] + [H] = [H]_{\text{tot}} \tag{5}$$

$$[HG_1] + [G_1] = [\text{G}_1]_{\text{tot}} \tag{6}$$

$$[HG_2] + [G_2] = [\text{G}_2]_{\text{tot}} \tag{7}$$

$$K_{a1} = \frac{[HG_1]}{[\text{H}] \times [G_1]} \tag{8}$$

$$K_{a2} = \frac{[HG_2]}{[\text{H}] \times [G_2]} \tag{9}$$

This approach was used to study the complexation behavior of compounds **7** and **8** toward Br$^-$, I$^-$, ClO_4^-, HSO_4^-, and NO_3^- (in all cases Bu_4N^+ salts) in MeCN; the K_a values are summarized in Table 4 and typical examples of the spectra are shown in Figure 3. The K_a values for the complexation of Br$^-$, I$^-$, and ClO_4^- were also determined with ITC and are in excellent agreement. The data for ClO_4^- complexation clearly demonstrate the validity of this method, even for very low K_a values.

Table 4. K_a values in MeCN of different complexes of compounds **7** and **8** obtained with the ESI-MS competitive method.

Complexes[a]	K_a[b] [M^{-1}]	Complexes[a]	K_a[b] [M^{-1}]
7@Br$^{-[c]}$	680 (680)	**8**@Br$^{-[c]}$	680 (670)
7@I$^-$	170 (175)	**8**@I$^-$	180 (173)
7@ClO_4^-	39 (37)	**8**@ClO_4^-	40 (35)
7@HSO_4^-	1700	**8**@HSO_4^-	1680
7@NO_3^-	870	**8**@NO_3^-	880

[a] **7**@Br$^-$ and **8**@Br$^-$ were used as the reference complexes. [b] ITC data are in parentheses. [c] The corresponding Cl$^-$ complexes were used as reference complexes.

The possible influence of a change of the settings of the mass spectrometer on the ratio of the peak intensities of the complexes **7**@Cl$^-$ and **7**@Br$^-$ (and consequently on the K_a value) was studied. Varying the capillary voltage from 110 eV to 50 eV caused significant changes in the intensities of the complexes; however, the ratio remained the same (only a variation of 5%). This means that the influence on the K_a value is negligible.

This method has also been validated by studying the influence of variations in the concentrations of the competing anions. The results in triplicate are summarized in Table 5 for the competitive titration of **8**@I$^-$ with different concentrations of Bu_4NBr. Table 5 clearly shows the reproducibility within one set of experiments, but it also makes clear that changing the concentration hardly influences the K_a value. The absolute values of the intensities in **8**@Br$^-$ in entries 2 and 3 in Table 5 are almost the same, despite the larger Br$^-$ concentration in entry 3. This is caused by suppression (vide supra); however, because the intensity of **8**@I$^-$ also drops, the ultimate K_a value is unaffected.

The ESI-MS competitive method has also been successfully applied to determine the K_a values of the anion complexation behavior of the glucose- and galactose-functionalized thiourea cavitands **11** and **12** toward Br$^-$, I$^-$, HSO_4^-, and

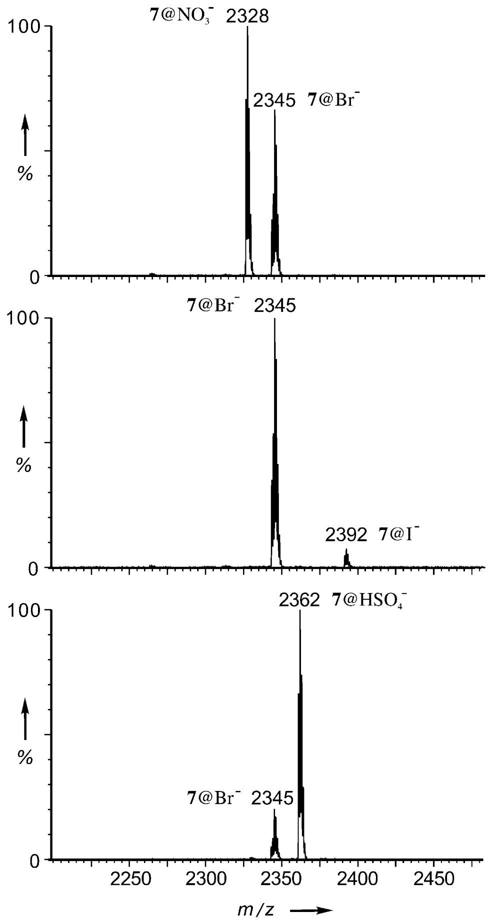


Figure 3. ESI-MS spectra in MeCN of mixtures of the complexes: **7**@Br$^-$ with a) **7**@NO$_3^-$, b) **7**@I$^-$, c) **7**@HSO$_4^-$. Initial concentrations: **7** (0.2 mM), Bu_4NBr (0.4 mM), Bu_4NNO_3 (0.4 mM), Bu_4NI (0.4 mM), Bu_4NHSO_4 (0.4 mM).

Table 5. Competitive titration of **8**@I$^-$ with Bu_4NBr.

Entry	Concentration [mM]		Run	Intensity		$\frac{\sqrt{I(\mathbf{8}@\ \mathrm{Br}^-)}}{\sqrt{I(\mathbf{8}@\ \mathrm{I}^-)}}$	K_a(**8**@I$^-$) [M^{-1}]
	Bu_4NBr	Bu_4NI		**8**@Br$^-$	**8**@I$^-$		
1	0.2	0.4	a	60322	18287	1.8162	172.9
			b	60977	18653	1.8080	173.7
			c	61231	18562	1.8166	172.8
2	0.4	0.4	a	81919	6111	3.6613	172.7
			b	81435	6093	3.6558	173.0
			c	80289	6132	3.6185	174.8
3	0.6	0.4	a	80458	2807	5.3538	178.4
			b	81313	2803	5.3860	177.3
			c	80425	2819	5.3413	178.8

NO_3^- in a 1:1 mixture of MeCN and water. The results are summarized in Table 6.

Evaluation of the K_a values: The complexation behavior of the acetylated cavitands **7** and **8** and their deacetylated analogues **11** and **12** toward different anions was studied. There is a selectivity for Cl$^-$ in all cases. For compounds **7** and **8** (in MeCN), the binding affinities follow the order: $Cl^- > HSO_4^- > NO_3^- > Br^- > I^- > ClO_4^-$ (Table 4). As expected, in a 1:1 MeCN/water mixture, the K_a values are much lower. For compounds **11** and **12**, the K_a values follow almost the same order: $Cl^- > HSO_4^- > Br^- > NO_3^- > I^-$ (Table 6). For the Cl$^-$ complexation of compounds **7**, **8**, and **9**, thermodynamic data have been determined with ITC (Table 1). The complexation is enthalpically driven, but the entropy factor also favors complexation. In the cases of compounds **7** and **8**, there is a Cl$^-$/Br$^-$ selectivity of ≈22. Surprisingly, this selectivity is much more pronounced than that found for the corresponding thioureamethyl cavitands that do not contain sugar moieties, such as **20** in $CDCl_3$. The preference for Cl$^-$ has also been found in other studies.[7c,29] The Br$^-$ and I$^-$ ions are more weakly complexed because of their "softer" nature, despite their increased size. Presumably, the spherical Cl$^-$ has a better fit within the thiourea binding pocket than the trigonal NO_3^- and tetrahedral HSO_4^-.

Table 6. K_a values of different complexes of compounds **11** and **12** in MeCN/water = 1:1 obtained with the ESI-MS competitive method.

Complexes	$K_a^{[a]}$ [M^{-1}]	Complexes	$K_a^{[b]}$ [M^{-1}]^1
11@Br$^-$	162	**12**@Br$^-$	157
11@I$^-$	83	**12**@I$^-$	78
11@HSO$_4^-$	173	**12**@HSO$_4^-$	176
11@NO$_3^-$	114	**12**@NO$_3^-$	109

[a] Reference complex **11**@Cl$^-$ (K_a = 260 M^{-1}, determined by ITC). [b] Reference complex **12**@Cl$^-$ (K_a = 250 M^{-1}, determined by ITC).

The presence of a glucose or a galactose moiety hardly influences the K_a value for the Cl$^-$ complexation (in MeCN). The K_a value of propylthioureamethyl cavitand **20** is 15400 M^{-1}, compared to 14900 M^{-1} for the corresponding pentyl chain-containing acetylated glucose thioureamethyl cavitand **9** (Table 3). However, probably for steric reasons, the cellobiose unit has a negative effect on the complexation. Comparing the values for the Cl$^-$ complexation of acetylated **7** and **10** and deacetylated **11** and **14**, the K_a values drop from 15100 to 4600 M^{-1} (in MeCN) and from 250 to 100 M^{-1} (in MeCN/water = 1:1), respectively.

Our study clearly shows the positive effect of bringing together ligating sites on a molecular platform. For instance, compound **7** binds Cl$^-$ in MeCN about 33 times better than the acyclic analogue **17** (K_a = 440 M^{-1}). The corresponding deacetylated compounds **11** and **18** give K_a values of 250 and ≈5 M^{-1} for Cl$^-$ complexation in MeCN/water (1:1).

Despite the absence of a thiourea moiety, the thiamethyl-linked glucose-containing cavitands **15** and **16** exhibit a reasonable complexation of Cl$^-$, namely, K_a values of 5200 (MeCN) and ≈60 M^{-1} (MeCN/water = 1:1), respectively. In the former case, the K_a value is only three times lower than that of the corresponding thiourea cavitand **7**. Apparently, the combination of a cavitand cavity and glucose units facilitates Cl$^-$ complexation.

Conclusion

In this study we have demonstrated the value of ESI-MS for the determination of K_a values for anion complexation with (acetylated) glycocluster thioureamethyl cavitands. The linear relationship found between the square root of the intensity and the concentration of the formed host–guest complex, allows direct determination of the K_a value by means of a titration experiment. However, this linearity is only observed when the suppression, the competitive influence of the titrant, is negligible. To avoid the possible influence of suppression, competitive methods were elaborated, namely, the "calibrated curve competitive method" and the "competitive method". The latter method is not sensitive to suppression and does not require a calibration curve. It is based on the comparison of the square roots of intensities of different related host–guest complexes. It is an excellent method for the rapid quantitative determination of the complexation behavior of a host toward a variety of guests.

The (acetylated) glycocluster thioureamethyl cavitands show a high affinity for chloride in MeCN. The K_a values for compounds **7**–**9** is $\approx 1.5\times10^4\,\text{M}^{-1}$, which is more than 20 times higher than the corresponding values for the complexation of bromide. In general, the affinity of cavitand-based anion receptors for anions is 30 times higher than that of the simple analogues **17** and **18** in both MeCN and MeCN/water (1:1).

We feel that the described ESI-MS-based methods are very valuable, new tools for K_a value determinations. They are a useful alternative when other methods of determination fail.

Experimental Section

General: The reagents used were purchased from Aldrich or Acros Chimica and used without further purification. All the reactions were performed under a dry argon atmosphere. All solvents were freshly distilled before use. Dry pyridine was obtained by distillation over calcium hydride. Melting points were measured on a Sanyo Gellenkamp Melting Point Apparatus and are uncorrected. Proton and carbon NMR spectra were recorded on a Varian Unity Inova (300 MHz) spectrometer. Residual solvent protons were used as an internal standard and chemical shifts are given relative to tetramethylsilane (TMS). Chromatography was performed with silica gel (SiO_2, Merck, 0.063–0.2 mm). MALDI spectra were recorded on a Voyager-DE RP Biospectrometry Workstation by PerSeptive Biosystems, Inc. (accelerating voltage 20 000 V, mode of operation: reflector, polarity: positive, matrixes: dithranol and DHB). Compounds **1**,[23] **2**,[30a] **4**,[30a] **6**,[30a] **17**,[30b] **18**,[30b] and **19**[30c] were prepared following literature procedures.

ESI-MS: The ESI-MS experiments were carried out with a Micromass LST ESI-TOF instrument. The solutions were introduced at a flow rate of 20 μL min^{-1} for 2 min (120 scans). The standard spray conditions, unless otherwise specified, were: capillary voltage 2500 V, sample cone voltage 30–110 V (optimal conditions for compounds **7**–**10** 80 V, and for **11**–**14** 30 V), desolvation gas flow 250 L h^{-1}, source temperature 100 °C, desolvation temperature 100 °C, extraction cone voltage ~0 V and flow cone gas is maintained at about 0 L h^{-1}. During a set of measurements, the distance between the capillary and the cone is kept constant. Every solution was injected five times. The values of the three intensities that are the closest to one another were used for the calculations. To check the stability of the signal, one of the solutions was used as a reference; it was injected in between the five injections of each solution. The intensity of the signal of the complex was calculated as the sum of the intensities of all the components of the isotopic pattern.

ITC: The calorimetric titration experiments were performed in a Microcal VP-ITC microcalorimeter with a cell volume of 1.4115 mL. The final curves were modeled by means of a nonlinear regression analysis. The fittings were calculated with Microcal Origin software.

Solubility measurements: A suspension of the relevant compound in water (or acetonitrile) was sonicated for 20 min at 25 °C. The solid was filtered off and the resulting solution (1–5 mL) was evaporated to dryness (a freeze dryer was used in the case of water). The experiments were repeated twice.

Calculations: The system of Equations (4)–(9) was numerically solved with the Maple 8 program (Waterloo Maple Inc.). Calibration curve data were also treated with the Maple 8 program based on the system of Equations (10)–(14) that include binding constant Equations (10) and (11) and mass balance Equations (12)–(14) (known parameters: $K_{\text{a,ref}}$ = binding constant of the reference host–guest complex; $[G_{\text{ref}}]_{\text{tot}}$, $[H]_{\text{tot}}$, $[G_{\text{new}}]_{\text{tot}}$ = total concentration of the reference guest, host, and the new guest added, respectively; $[HG_{\text{ref}}]$ = concentration of the reference host–guest complex derived from the calibration curve; unknowns: $K_{\text{a,new}}$ = binding constant of the host with new guest; $[G_{\text{ref}}]$, $[G_{\text{new}}]$, $[H]$, $[HG_{\text{new}}]$ = concentrations of the reference guest, new guest, host, and the complex of the host with the new guest, respectively):

$$K_{\text{a,ref}} = \frac{[HG_{\text{ref}}]}{[H][G_{\text{ref}}]} \tag{10}$$

$$K_{\text{a,new}} = \frac{[HG_{\text{new}}]}{[H][G_{\text{new}}]} \tag{11}$$

$$[G_{\text{ref}}]_{\text{tot}} = [HG_{\text{ref}}] + [G_{\text{ref}}] \tag{12}$$

$$[G_{\text{new}}]_{\text{tot}} = [HG_{\text{new}}] + [G_{\text{new}}] \tag{13}$$

$$[H]_{\text{tot}} = [HG_{\text{ref}}] + [HG_{\text{new}}] + [H] \tag{14}$$

Equation (15) represents the analytical solution of the system of Equations (10)–(14), which is more convenient for the analysis of an array of data. The calculations were performed with Microsoft Excel [Eq. (15)].

$$K_{\text{a,new}} = \frac{K_{\text{a,ref}}([G_{\text{ref}}]_{\text{tot}}-[HG_{\text{ref}}])([H]_{\text{tot}}-[HG_{\text{ref}}])-[HG_{\text{ref}}]}{K_{\text{a,ref}}([G_{\text{ref}}]_{\text{tot}}-[HG_{\text{ref}}])([G_{\text{new}}]_{\text{tot}}-[H]_{\text{tot}} + [HG_{\text{ref}}]) + [HG_{\text{ref}}]} \times \frac{K_{\text{a,ref}}([G_{\text{ref}}]_{\text{tot}}-[HG_{\text{ref}}])}{[HG_{\text{ref}}]} \tag{15}$$

Tetrakis(phthalimidomethyl)tetramethyl cavitand (3): A mixture of tetrakis(bromomethyl)tetramethyl cavitand **1** (0.96 g, 1 mmol) and potassium phthalimide (1.11 g, 6 mmol) in MeCN (50 mL) was refluxed for five days. The reaction mixture was evaporated to dryness, whereupon CH_2Cl_2 (50 mL) and 1 N NaOH (25 mL) were added to the residue. After the mixture had been stirred for 10 min, the organic layer was separated and washed with 1 N NaOH (2×25 mL) and water (25 mL), dried over $MgSO_4$, and evaporated to dryness. The resulting solid was purified by column chromatography (CH_2Cl_2 containing 2.5 % MeOH). Yield: 65 %; m.p. > 350 °C (CH_2Cl_2/EtOAc); ^{1}H NMR ($CDCl_3$): δ = 7.93–7.78 (m, 8H; Phth), 7.78–7.63 (m, 8H; Phth), 7.20 (s, 4H; ArH), 5.81 (d, J = 7.3 Hz, 4H; O_2CH), 4.92 (q, J = 7.3 Hz, 4H; Ar_2CH), 4.43 (d, J = 7.3 Hz, 4H; O_2CH), 4.66 (s, 8H; $ArCH_2N$), 1.68 ppm (d, J = 7.3 Hz, 12H; CH_3C); ^{13}C NMR ([D$_6$]DMSO): δ = 168.0, 153.5, 138.8, 134.0, 123.4, 132.1, 121.1, 119.7, 99.6, 32.7, 31.1, 16.1 ppm; MS (MALDI): m/z (%): 1228.9 (100) $[M]^+$; elemental analysis calcd (%) for $C_{72}H_{52}N_4O_{16}$ (1229.2): C 70.35, H 4.26, N 4.56; found: C 70.07, H 4.18, N 4.83.

Tetrakis(aminomethyl)tetramethyl cavitand (5): A mixture of tetrakis(phthalimidomethyl)tetramethyl cavitand **3** (2.9 g, 2.36 mmol) and hydrazine hydrate (2.95 g) in MeOH/CH_2Cl_2 (1:3, 200 mL) was refluxed for 26 h. The solid was filtered off, the solution was evaporated to dryness, and the crude product was triturated with diethyl ether (3×30 mL). Double recrystallization from EtOH/*i*PrOH gave pure **5** as a white powder. Yield: 60 %; m.p. > 350 °C (EtOH/*i*PrOH); ^{1}H NMR ([D$_6$]DMSO): δ = 7.61 (s, 4H; ArH), 5.89 (d, J = 7.3 Hz, 4H; O_2CH), 4.80 (q, J = 7.7 Hz, 4H; Ar_2CH), 4.41 (d, J = 7.7 Hz, 4H; O_2CH), 3.46 (s, 8H; $ArCH_2N$), 1.79 ppm (d, J = 7.7 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ = 152.1, 138.7, 129.3, 119.6, 99.4, 35.2, 31.2, 16.1 ppm; MS (MALDI): m/z (%): 708.5 (100) [M]$^+$, 731.5 (50) [M+Na]$^+$); elemental analysis calcd (%) for $C_{40}H_{44}N_4O_8$ (708.8): C 67.78, H 6.26, N 7.90; found: C 67.94, H 6.50, N 7.71.

General procedure for the preparation of the acetylated glycoclusters 7–10: A solution of a pyranosylisothiocyanate[31] (1.06 mmol) and tetrakis(aminomethyl) cavitand **5** or **6** (0.21 mmol) in pyridine (20 mL) was stirred at room temperature for 3 d. After evaporation of the solvent, the resulting solid was triturated with diethyl ether (2 × 20 mL) and subsequently recrystallized from 2-propanol. The pyranosylisothiocyanates used for this synthesis (tetra-*O*-acetyl-β-D-glucopyranosylisothiocyanate,[31a] tetra-*O*-acetyl-β-D-galacto-pyranosylisothiocyanate[31b] and hepta-*O*-acetyl-β-D-cellobiosyl-isothiocyanate[31c]) were prepared by a slightly modified procedure used for the synthesis of the glucose derivative[31a] (refluxing the corresponding α-bromopyranoside with $Pb(NCS)_2$ in toluene for 4–6 h).

Tetrakis(1-tetraacetylglucosylthioureidomethyl)tetramethyl cavitand (7): Yield: 80%; m.p. 230–231 °C (2-propanol); ^1H NMR ([D_6]DMSO): δ = 7.73 (brs, 12H; ArH, NH), 5.91 (d, J = 7.0 Hz, 4H; O_2CH), 5.78 (brs, 4H; H(1)-glu), 5.33 (t, J = 9.3 Hz, 4H; H(3)-glu), 5.0–4.7 (m, 12H; H(2), H(4)-glu, Ar_2CH), 4.43 (brs, 8H; $ArCH_2$), 4.25 (brs, 4H; O_2CH), 4.15 (dd, J(6a,6b) = 12.0 Hz, J(6a,5) = 4.0 Hz, 4H; H(6a)-glu), 3.95 and 3.92 (brs + s, 8H; H(6b), H(5)-glu), 1.97 (brs, 36H; CH_3CO), 1.93 (s, 12H; CH_3CO), 1.81 ppm (d, J = 7.0 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ = 183.1, 169.9, 169.4, 169.2, 152.5, 139.0, 123.3, 121.3, 99.2, 81.4, 72.7, 71.9, 70.4, 67.9, 61.6, 38.1, 31.3, 20.2, 20.2, 15.9 ppm; MS (MALDI): m/z (%): 2265.1 (100) [M]$^+$, 2288.1 (80) [M+Na]$^+$; elemental analysis calcd (%) for $C_{100}H_{120}N_8O_{44}S_4$ (2266.4): C 53.00, H 5.34, N 4.94, S 5.66; found: C 52.73, H 5.59, N 4.86, S 5.62.

Tetrakis(1-tetraacetylgalactosylthioureidomethyl)tetramethyl cavitand (8): Yield: 85%; m.p. 233 °C (2-propanol); ^1H NMR ([D_6]DMSO): δ = 7.73 (brs, 12H; ArH, NH), 5.91 (d, J = 7.5 Hz, 4H; O_2CH), 5.78 (t, J = 9.5 Hz, 4H; H(1)-gal), 5.38–5.18 (m, 8H; H(4), H(3)-gal), 4.93 (t, J = 9.2 Hz, 4H; H(2)-gal), 4.81 (d, J = 7.5 Hz, 4H; Ar_2CH), 4.44 (brs, 8H; $ArCH_2$), 4.22 (t + brs, J = 6.2 Hz, 8H; O_2CH, H(5)-gal), 3.98 and 3.97 (s, 8H; H(6a), H(6b)-gal), 2.09 (s, 12H; CH_3CO), 1.99 (s, 12H; CH_3CO), 1.90 (s, 24H; CH_3CO), 1.82 ppm (d, J = 7.3 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ = 183.2, 169.9, 169.4, 169.3, 152.5, 139.0, 123.1, 121.8, 99.1, 81.4, 72.7, 71.9, 70.4, 67.9, 61.6, 37.8, 31.3, 20.2, 20.3, 15.9 ppm; MS (MALDI): m/z (%): 2265.0 (100) [M]$^+$, 2288.0 (80) [M+Na]$^+$; elemental analysis calcd (%) for $C_{100}H_{120}N_8O_{44}S_4$ (2266.4): C 53.00, H 5.34, N 4.94, S 5.66; found: C 52.80, H 5.50, N 4.83, S 5.60.

Tetrakis(1-tetraacetylglucosylthioureidomethyl)tetraamyl cavitand (9): Yield: 72%; m.p. 179–180 °C (2-propanol); ^1H NMR ([D_6]DMSO): δ = 7.77 (brs, 8H; NH), 7.58 (s, 4H; ArH), 5.91 (d, J = 7.3 Hz, 4H; O_2CH), 5.82 (brs, 4H; H(1)-glu), 5.34 (t, J = 9.4 Hz, 4H; H(3)-glu), 4.95–4.7 (m, 8H; H(2), H(4)-glu), 4.60 (t, J = 7.9 Hz, 4H; Ar_2CH), 4.43 (brs, 8H; $ArCH_2$), 4.28 (brd, 4H; O_2CH), 4.15 (dd, J(6a,6b) = 12.4 Hz, J(6a,5) = 4.4 Hz, 4H; H(6a)-glu), 4.1–3.7 (brs + s, 8H; H(6b), H(5)-glu), 2.35 (brs, 8H; CH_2CH), 1.98 and 1.97 (s, 36H; CH_3CO), 1.93 (s, 12H; CH_3CO), 1.45–1.2 (brm, 24H; $(CH_2)_3$), 0.88 ppm (d, J = 7.0 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ = 183.2, 169.9, 169.4, 169.2, 152.9, 138.0, 123.4, 121.7, 99.1, 81.4, 72.7, 71.9, 70.5, 68.0, 61.6, 38.1, 36.9, 31.4, 29.2, 27.4, 22.1, 20.2, 20.3, 13.9 ppm; MS (MALDI): m/z (%): 2489.1 (70) [M]$^+$, 2512.1 (100) [M+Na]$^+$; elemental analysis calcd (%) for $C_{116}H_{152}N_8O_{44}S_4$ (2490.8): C 55.94, H 6.15, N 4.50, S 5.15; found: C 56.07, H 6.25, N 4.26, S 5.07.

Tetrakis(1-tetracellobiosylthioureidomethyl)tetramethyl cavitand (10): Yield: 86%; m.p. 248–250 °C (2-propanol); ^1H NMR ([D_6]DMSO): δ = 7.74 (brs, 12H; ArH, NH), 5.91 (brs, 4H; O_2CH), 5.78 (t, J = 8.4 Hz, 4H; H(1)-cel), 5.35–5.1 (m, 8H; H1″, H(3)-cel), 5.0–4.7 (m, 16H; Ar_2CH, H(2), H(2)″, H(3)″-cel), 4.65 (t, J = 9.0 Hz, 4H; H(4)″-cel), 4.42 (brs, 8H; $ArCH_2$), 4.25 (t + brs, J = 10.8 Hz, 8H, O_2CH, H(4)-cel), 4.14–3.9 (m, 16H; H(6a), H(6a)″, H(6b), H(6b)″-cel), 3.7–3.9 (m, 8H; H_5, H_5″-cel), 2.05 (s, 12H; CH_3CO), 2.02 (s, 12H; CH_3CO), 2.0–1.94 (m, 48H; CH_3CO), 1.92 (s, 12H; CH_3CO), 1.82 ppm (d, J = 6.6 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ = 183.2, 170.2, 169.9, 169.5, 169.4, 169.3, 169.1, 168.9, 152.5, 139.0, 123.3, 121.3, 99.2, 81.2, 76.2, 73.1, 72.6, 72.2, 71.0, 70.6, 70.4, 67.7, 62.2, 61.5, 38.0, 31.3, 20.6, 20.4, 20.3, 20.1, 15.9 ppm; MS (MALDI): m/z (%): 3438.5 (100) [M+Na]$^+$; elemental analysis calcd (%) for $C_{149}H_{186}N_8O_{75}S_4$ (3417.4): C 52.37, H 5.49, N 3.28, S 3.75; found: C 52.31, H 5.53, N 3.16, S 3.79.

General procedure for the preparation of the glycoclusters 11, 12, 13, 14, 16: A catalytic amount of MeONa (≈5% with respect to each AcO-substituent in the precursor) was added to a solution of the acetylated glycoclusters **7**, **8**, **9**, **10**, **15** (0.088 mmol) in MeOH/CH_2Cl_2 (1:4, 50 mL). The reaction mixture was stirred overnight at room temperature. Water (5 mL) was added and the reaction mixture was stirred for an additional 20 min. Subsequently, Amberlite IR-120 H$^+$ (washed before with methanol) was added and stirring was continued for an additional 15 min. The Amberlite was filtered off and the organic solvents were evaporated at reduced pressure (t < 30 °C); the water was removed by freeze-drying. The yield of glycoclusters is almost quantitative. An additional purification by sonication with diisopropyl ether and washing with isopropanol is also possible.

Tetrakis(1-glucosylthioureidomethyl)tetramethyl cavitand (11): Yield: 96%; m.p. > 350 °C; ^1H NMR ([D_6]DMSO): δ = 7.73 (s, 4H; ArH), 7.67 (s, 4H; NH), 7.49 (s, 4H; NH), 5.95 (d, J = 6.6 Hz, 4H; O_2CH), 5.16 (brs, 4H; H(1)-glu), 4.81 (q, J = 7.3 Hz, 4H; Ar_2CH), 4.43 (brs, 8H; $ArCH_2$), 4.30 (d, J = 6.6 Hz, 4H; O_2CH), 3.97 (brs, 16H; OH), 3.59 (brd, J_2 = 11.3 Hz, 4H, H(6a)-glu), 3.42 (brd, J_2 = 10.0 Hz, 4H; H(6b)-glu), 3.22–2.85 (m, 16H; H(2), H(3), H(4), H(5)-glu), 1.81 ppm (d, J = 7.3 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ = 183.1, 152.5, 139.0, 123.8, 121.1, 99.1, 83.6, 78.0, 77.5, 72.8, 69.7, 60.5, 37.8, 31.3, 15.9 ppm; MS (MALDI): m/z (%): 1615.8 (100) [M+Na]$^+$; elemental analysis calcd (%) for $C_{68}H_{88}N_8O_{28}S_4$ (1593.7): C 51.25, H 5.57, S 8.05; found: C 50.98, H 5.61, S 8.00.

Tetrakis(1-galactosylthioureidomethyl)tetramethyl cavitand (12): Yield: 95%, m.p. > 350 °C; ^1H NMR ([D_6]DMSO): δ = 7.23 (s, 4H; ArH), 7.67 (s, 4H; NH), 7.49 (s, 4H; NH), 5.95 (d, J = 6.6 Hz, 4H; O_2CH), 5.15 (brs, 4H; H(1)-gal), 4.98 (brs, 8H; OH), 4.81 (brd, J = 7.3 Hz, 8H; Ar_2CH, OH), 4.42 (brs, 12H; $ArCH_2$, OH), 4.29 (d, J = 6.6 Hz, 4H; O_2CH), 3.58 (d, J_2 = 11.3 Hz, 4H; H(6a)-gal), 3.5–2.8 (m, 20H; H(2), H(3), H(4), H(5), H(6b)-gal), 1.81 ppm (d, J = 7.3 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ = 183.0, 152.5, 139.0, 123.8, 121.1, 99.1, 83.5, 78.0, 69.7, 72.8, 77.5, 60.5, 37.8, 31.3, 15.9 ppm; MS (MALDI): m/z (%): 1615.6 (100) [M+Na]$^+$; elemental analysis calcd (%) for $C_{68}H_{88}N_8O_{28}S_4$ (1593.7): C 51.25, H 5.57, S 8.05; found: C 50.95, H 5.67, S 7.98.

Tetrakis(1-glucosylthioureidomethyl)tetraamyl cavitand (13): Yield: 90%; m.p. > 350 °C; ^1H NMR ([D_6]DMSO): δ = 7.72 (s, 4H; NH), 7.58 (s, 4H; ArH), 7.48 (s, 4H; NH), 5.95 (d, J = 6.6 Hz, 4H; O_2CH), 5.19 (brs, 4H; H(1)-glu), 4.96 (brs, 8H; OH), 4.82 (brs, 4H; OH), 4.62 (q, J = 7.3 Hz, 4H; Ar_2CH), 4.43 (brs, 8H; $ArCH_2$), 4.30 (d, J = 6.6 Hz, 4H; O_2CH), 3.60 (d, J_2 = 11.0 Hz, 4H; H(6a)-glu), 3.46 (d, J_2 = 10 Hz, 4H; H(6b)-glu), 3.4–2.9 (m, 20H; OH, H(2), H(3), H(4), H(5)-glu), 2.37 (q, J = 7.2 Hz, 8H; CH_2CH), 1.52–1.15 (m, 24H; $(CH_2)_3$), 0.89 ppm (t, J = 7.2 Hz, 12H; CH_3); ^{13}C NMR ([D_6]DMSO): δ = 183.1, 152.8, 138.0, 123.8, 121.5, 99.2, 83.6, 78.0, 77.5, 72.8, 69.7, 60.5, 37.8, 36.9, 31.4, 29.2, 27.4, 22.2, 13.9 ppm; MS (MALDI): m/z (%): 1840.0 (100) [M+Na]$^+$; elemental analysis calcd (%) for $C_{84}H_{120}N_8O_{28}S_4$ (1818.2): C 55.49, H 6.65, S 7.05; found: C 55.28, H 6.75, S 6.95.

Tetrakis(1-cellobiosylthioureidomethyl)tetramethyl cavitand (14): Yield: 92%; m.p. > 350 °C; ^1H NMR ([D_6]DMSO): δ = 7.73 (s, 8H; ArH, NH), 7.51 (s, 4H; NH), 5.96 (brs, 4H; O_2CH), 5.21 (brs, 4H; H(1)-cel), 4.81 (d, J = 6.9 Hz, 8H; Ar_2CH, OH), 4.6–2.9 (brs + m, 88 H + water; $ArCH_2$, O_2CH, OH, H(2,6b), H(1″,6b″)-cel), 1.81 ppm (d, J = 6.3 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ = 180.9, 152.5, 139.0, 123.8, 121.1, 103.0, 83.3, 79.7, 76.7, 76.4, 76.2, 75.7, 74.8, 73.3, 72.5, 70.0, 61.0, 60.2, 37.8, 31.3, 15.9 ppm; MS (MALDI): m/z (%): 2263.9 (100) [M+Na]$^+$; elemental analysis calcd (%) for $C_{92}H_{128}N_8O_{48}S_4 \cdot 10H_2O$ (2422.5): C 45.62, H 6.16, N 4.63, S 5.29; found: C 45.82, H 6.04, N 4.43, S 5.18.

Tetrakis(1-glucosylthiomethyl)tetramethyl cavitand (16): Yield: 91%; m.p. > 350 °C; ^1H NMR ([D_6]DMSO): δ = 7.63 (s, 4H; ArH), 6.02 (d, J = 7.3 Hz, 4H; O_2CH), 5.04 (brs, 8H; OH), 4.79 (q, J = 7.3 Hz, 4H; Ar_2CH), 4.70 (brs, 4H; OH), 4.40–4.20 (m, 8H; O_2CH, H(1)-glu), 3.85–3.67 (m, 8H; H(6a)-glu, OH), 3.5–3.25 (m, 12H; H(6b)-glu, $ArCH_2S$), 3.25–3.0 (m, 12H; H(3), H(4), H(5)-glu), 2.93 (t, J = 9.0 Hz, 4H; H(2)-glu) 1.82 ppm (d, J = 7.3 Hz, 12H; CH_3C); ^{13}C NMR ([D_6]DMSO): δ =

152.1, 138.8, 125.9, 120.0, 99.2, 84.3, 81.0, 78.2, 73.0, 70.2, 31.2, 21.9, 16.0 ppm; MS (MALDI): *m*/*z* (%): 1447.6 (100) $[M{+}\mathrm{Na}]^+$; elemental analysis calcd (%) for $C_{64}H_{80}O_{28}S_4$ (1425.6): C 53.92, H 5.66, S 9.00; found: C 53.63, H 5.77, S 8.89.

Tetrakis(1-tetraacetylglucosylthiomethyl)tetramethyl cavitand (15): A solution of 2,3,4,6-tetra-*O*-acetyl-*β*-glucopyranosylthiol[32] (530 mg, 1.45 mmol) in CH_2Cl_2 (5 mL) was added to a suspension of K_2CO_3 (0.95 g, 2.9 mmol) in a solution of tetrakis(bromomethyl)tetramethyl cavitand (**1**, 289 mg, 0.29 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred at room temperature for 75 h, whereupon the salts were filtered off. The solution was evaporated to dryness at reduced pressure ($T < 40\,°C$). The solid was triturated with diethyl ether and recrystallized from MeOH. Yield: 88 %; m.p. 176–177 °C (MeOH); ^1H NMR ($CDCl_3$): $\delta = 7.20$ (s, 4H; ArH), 5.91 (d, $J = 7.3$ Hz, 4H; O_2CH), 5.19 (t, $J = 9.2$ Hz, 4H; H(3)-glu), 5.09 (t, $J = 9.5$ Hz, 4H; H(4)-glu), 5.00 (t, $J = 9.9$ Hz, 4H; H(2)-glu), 4.98 (q, $J = 7.3$ Hz, 4H, Ar_2CH), 4.55 (d, $J = 9.9$ Hz, 4H; H(1)-glu), 4.36 (d, $J = 7.3$ Hz, 4H; O_2CH), 4.24 (dd, $J(6a,6b) = 12.5$ Hz, $J(5,6b) = 4.4$ Hz, 4H; H(6b)-glu), 4.10 (dd, $J(6b,6a) = 12.5$ Hz, $J(5,6a) = 2.4$ Hz, 4H; H(6a)-glu), 3.6–3.9 (m, 12H; $ArCH_2$, H(5)-glu), 2.07 (s, 12H; CH_3CO), 2.04 (s, 12H; CH_3CO), 2.00 (s, 12H; CH_3CO), 1.81 (s, 12H; CH_3CO), 1.76 ppm (d, $J = 7.3$ Hz, 12H; CH_3C); ^{13}C NMR ($[D_6]$DMSO): $\delta = 169.9$, 169.4, 169.2, 168.9, 152.2, 138.6, 124.2, 120.5, 99.5, 82.5, 74.3, 72.9, 69.6, 68.1, 61.9, 31.2, 23.5, 20.3, 20.2, 19.6, 15.9 ppm; MS (MALDI): *m*/*z* (%): 2119.7 (80) $[M{+}\mathrm{Na}]^+$; elemental analysis calcd (%) for $C_{96}H_{112}O_{44}S_4$ (2098.2): C 54.96, H 5.38, S 6.11; found: C 54.80, H 5.38, S 6.06.

Tetrakis(propylthioureidomethyl)tetraamyl cavitand (20): A solution of propylisothiocyanate (242 mg, 0.25 mL, 1.8 mmol) and tetrakis(aminomethyl) cavitand **6** (278 mg, 0.3 mmol) in MeOH:CH_2Cl_2 (1/3; 20 mL) was stirred at room temperature for two days. The solvent was evaporated to dryness. The resulting solid was triturated with hexane (2 × 20 mL) and then purified by column chromatography (CH_2Cl_2 containing 5 % MeOH, $R_f = 0.17$) followed by trituration with MeCN (2 × 5 mL). Yield: 83 %; m.p. 214–215 °C; ^1H NMR ($CDCl_3$): $\delta = 7.12$ (s, 4H; ArH), 6.30 (br s, 4H; NH), 6.13 (br s, 4H; NH), 5.99 (d, $J = 7.2$ Hz, 4H; O_2CH), 4.73 (t, $J = 8.0$ Hz, 4H; Ar_2CH), 4.41 (d, $J = 7.2$ Hz, 4H; O_2CH), 4.35 (br s, 8H; $ArCH_2N$), 3.42 (br s, 8H; $AlkCH_2N$), 2.20 (q, $J = 7.2$ Hz, 8H; CH_2CH), 1.76–1.60 (m, 8H; $MeCH_2CH_2N$), 1.48–1.28 (m, 24H; $(CH_2)_3$), 1.02–0.86 ppm (m, 24H; CH_3); ^{13}C NMR ($CDCl_3$): $\delta = 181.0$, 153.1, 138.5, 122.8, 120.4, 99.9, 36.9, 32.0, 30.0, 27.6, 22.6, 22.2, 14.1, 11.5 ppm; MS (MALDI): *m*/*z* (%): 1336.7 (100) $[M]^+$; elemental analysis calcd (%) for $C_{72}H_{104}N_8O_8S_4$ (1337.9): C 64.64, H 7.83, N 8.38, S 9.59; found: C 64.67, H 7.82, N 8.29, S 9.64.

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