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Recognition of Anionic Carbohydrates by an Artificial Receptor in Water

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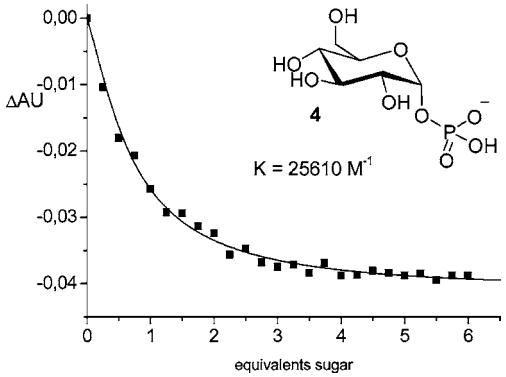
ABSTRACT



A tris-cationic artificial receptor 1 efficiently binds anionic carbohydrates even in aqueous solution as shown by NMR and UV titration experiments. Complex formation involves both ion pair formation and H-bonds to the sugar, explaining the preference for saccharides compared to simple anions and the observed selectivities among different sugars.

Artificial receptors that efficiently bind small biomolecules are interesting not only for the study of the underlying chemical principles of such molecular recognition events but also as examples for the design of chemosensors.[1] Finding such receptors for aqueous solvents is still challenging due to the strong dependence of most noncovalent interactions (such as H-bonds and electrostatic interactions) on solvent polarity and total ionic strength. Whereas much progress has been achieved in recent years for the binding of amino acids and small peptides in water,[2,3] the complexation of carbohydrates remains difficult.[4] Most artificial receptors reported so far only show efficient binding in organic solvents.[5] Only very few examples of carbohydrate recognition in aqueous solvents are known.[6] Additionally, the complex stabilities found in these cases are rather modest if covalent B−O bond formation in boronic acid-based binding motifs is neglected.[7] For example, Davis just recently reported a rigid tricyclic polyamide host that binds various carbohydrates with rather low affinities of $K \leq 30\ M^{-1}$ in water.[6a] Other receptors, including those studied earlier in work by Diederich[8] or Aoyama,[9] are also based on rigid preorganized hydrophobic cavities, which are, however, difficult to synthesize. We now want to report the efficient complexation of anionic carbohydrates by a flexible tris-cationic host with association constants $K > 10^3\ M^{-1}$ in water as shown by NMR and UV titration experiments.

We recently developed a C3-symmetric tris-cationic host **1** for the highly efficient binding of citrate in water ($K \approx$

(1) Reviews on chemosensors: (a) Martinez-Manez, R.; Sancenon, F. *Chem. Rev.* **2003**, *103*, 4419−4476. (b) Czarnik, A W.; Yoon, J. *Persp. Supramol. Chem.* **1999**, *4*, 177−191. (c) De Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, Terence E. *Chem.* Rev. **1997**, *97*, 1515−1566. (e) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423−428.

(2) For recent review articles on artificial peptide receptors, see: (a) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479−2494. (b) Schneider, H.-J. *Adv. Supramol. Chem.* **2000**, *6*, 185−216.

(3) For selected recent examples, see: (a) Schmuck, C.; Geiger, L. *J. Am. Chem. Soc.* **2004**, *126*, 8898−8899. (b) Schmuck, C.; Heil, M. *ChemBioChem* **2003**, *4*, 1232−1238. (c) Rensing, S.; Schrader, T. *Org. Lett.* **2002**, *4*, 2161−2164. (d) Jensen, K.; Braxmeier, T. M.; Demarcus, M.; Frey, J. G.; Kilburn, J. D. *Chem.−Eur. J.* **2002**, *8*, 1300−1309. (e) Xuo, R.; Greiveldinger, G.; Marenus, L. E.; Cooper, A.; Ellman, *J. A. J. Am. Chem. Soc.* **1999**, *121*, 4898−4899.

(4) For reviews, see: (a) Davis, A. P.; Wareham, R. S. *Angew. Chem.* **1999**, *111*, 3160−3179; *Angew. Chem., Int. Ed.* **1999**, *38*, 2978−2996. (b) Striegler, S. *Curr. Org. Chem.* **2003**, *7*, 81−102.

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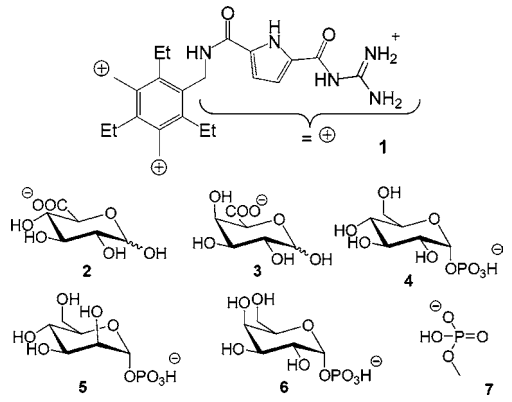
10^5 M^{-1}).[10] The same underlying aromatic tris-amine template has already been used, mainly by Mazik and coworkers, to construct neutral carbohydrate hosts by attaching aminopyridines to the amino groups[5b,c] or by Anslyn for boronic acid-based carbohydrate sensors.[11] However, monosaccharide binding by Mazik's neutral pyridine-based receptors is again restricted to organic solvents, whereas binding by boronic acids relies on covalent bond formation. We were therefore interested to test the carbohydrate binding affinity of our tris-cation **1** for anionic carbohydrates in more polar aqueous solvents. We chose glucuronic (**2**) and galacturonic acid (**3**) (both as anomeric mixtures), as well as the α-1-phosphates of glucose (**4**), mannose (**5**), and galactose (**6**), as substrates (Scheme 1). Methyl phosphate (**7**) was used as a nonsaccharide substrate for comparison.

Scheme 1. Receptor **1** and Substrates **2**−**7**



The binding affinities of **1** for the two uronic acids **2** and **3** were first analyzed by NMR titration experiments in 30% water in DMSO (v/v).[12] Aliquots of a stock solution of the corresponding uronic acetate (Na^+ salt) were added to a solution of the host (tris-chloride salt, $c_0 = 2$ mM for **2** and $c_0 = 0.5$ mM for **3**). The complexation-induced shift changes of the pyrrole CHs were recorded and the resulting binding isotherms analyzed by a nonlinear curve-fitting using a 1:1 complexation model (Figure 1). The 1:1 complex stoichiometry was independently confirmed by a Job plot. Glucuronic acid **2** binds with $K = 480$ M^{-1}, and galacturonic acid **3** binds more than three times stronger with $K = 1550$ M^{-1}. The better binding of galacturonic acid was also confirmed by UV titration studies in aqueous buffer solution (10 mM bis-tris buffer at pH = 6.0 in 20% water in DMSO). Under these conditions, **3** binds around two times better than **2** ($K = 6200$ versus 3200 M^{-1}, respectively).

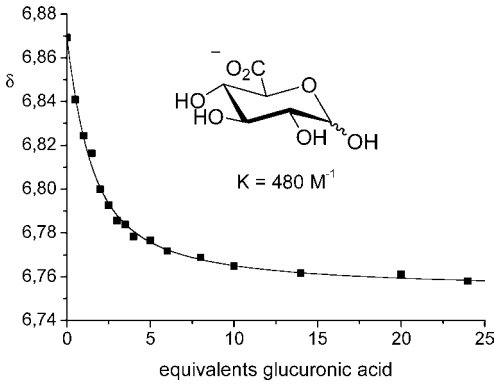


Figure 1. Binding isotherm for the complexation of glucuronic acid (**2**) by **1** as obtained from a NMR titration in 30% water in DMSO [based on the shift change of one of the pyrrole CHs].

These binding data suggest that also the C4−OH group of the sugar somehow interacts with the receptor. This is indeed supported by molecular modeling studies (Figure 2). According to these calculations (Macromodel 8.0, Amber* force field, GB/SA water solvation model, Monte Carlo conformational search with 50.000 steps),[13] for both sugars the carboxylate is simultaneously hydrogen bonded by all three cationic arms of the receptor, whereas the ring oxygen forms additional hydrogen bonds with one arm.[14] Interestingly, galacturonic acid **3** adopts a conformation in which the carboxylate as well as the OH at C1 and C3, which form intramolecular H-bonds to the carboxylate, are axial.[15] This

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(14) Binding by all three cationic arms is also supported by the fact that at pH = 7.4, where the receptor is only monoprotonated, no complexation of the uronic acids could be detected.

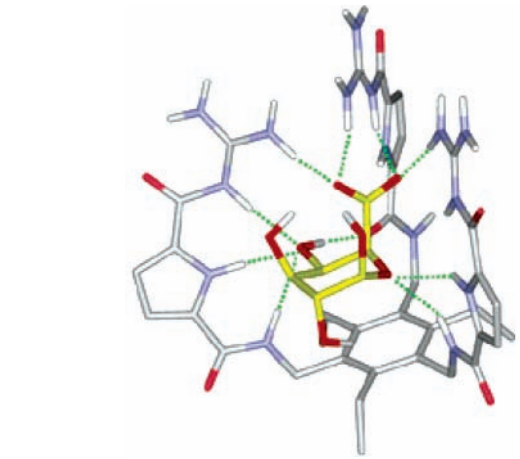

Figure 2. Calculated structure of the complex between receptor **1** (gray) and galacturonic acid **3** (yellow) showing the ion pair formation with the carboxylate (top) as well as the H-bonds to the ring oxygen (right) and the OH at C4 (left). Nonpolar hydrogens have been omitted for clarity.

conformation positions the now equatorial OH at C4 of galactose into a position that allows the formation of three additional hydrogen bonds with one of the receptor arms. In the complex with glucuronic acid (**2**) (not shown), the OH at C4 is in an axial position pointing away from these hydrogen bond donors. Hydrogen bond formation to this OH now requires a distortion of the glucose ring, which could explain the lower binding affinity of glucose.

This binding mode is in good agreement with the observed shift changes for the sugar CHs of **3** upon complex formation in DMSO. The shift changes for CH1 and CH4 ($\Delta\delta = 0.3$ and 0.11) are much larger than those for CH2, CH3, or CH5 ($\Delta\delta = 0.09$, 0.07, and 0.05, respectively). The larger downfield shifts of CH1 and CH4 could reflect the cationic hydrogen bonds to the ring oxygen atom and the C4−OH. Complex formation could also be detected by NOESY experiments (1:1 mixtures with $c = 10$ mM in DMSO at 25 °C). Unfortunately, the spectrum quality did not allow a detailled structure analysis due to the large degree of flexibility of the system and significant overlap between host and guest signals. However, distinct contacts could be assigned between the CH1 and the CH2 of the sugars and the pyrrole NH as well as the two amide NHs of the receptor. Cross-peaks between the pyrrole NH and the two amide NHs of the receptor furthermore indicate that the binding arms indeed adopt the conformation with all NHs pointing inward as needed for substrate binding.

For the sugar phosphates, we first determined the intrinsic binding affinities of the monoanions using UV titrations at pH = 4.0 (4 mM acetate buffer in water with 10% DMSO (v:v), $[\text{host}]_0 = 20\ \mu$M). This pH was chosen to ensure that only one ionic species of both substrates and host would be present in solution. At pH = 4, the host will be completely protonated, whereas the substrates will exist in form of the monoanions. Otherwise, at higher pHs, mixtures of different ratios of di- versus monoanions would be present for the various sugar phosphates for example. Furthermore, the host would be present as a mixture of fully protonated and partially deprotonated species. The binding of the phosphate monoanions at pH = 4 is extremely strong with association constants $K > 20\,000\ \text{M}^{-1}$ (Figure 3, Table 1). The affinity

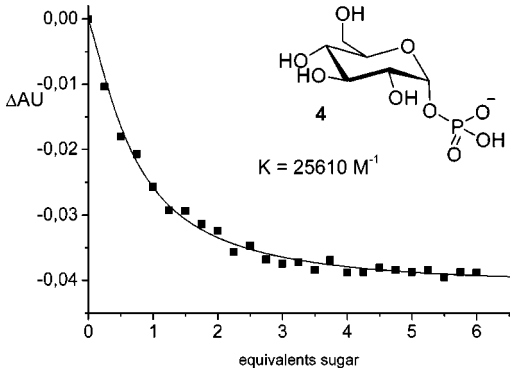

Figure 3. Binding isotherm for α-*D*-glucose-1-phosphate (**4**) as obtained from a UV titration in buffered water at pH = 4.0.

of host **1** toward the sugar phosphates is much larger than for the corresponding uronic acids. This is in good agreement with the stronger ion pair formed with a phosphate compared to a carboxylate. Also, at a physiological pH of 7.4, the phosphates still bind but with a much lower affinity of $K > 12\,000\ \text{M}^{-1}$ in 70% DMSO in water (10 mM bis-tris buffer, $[\text{host}]_0 = 25\ \mu$M). The weaker binding at pH = 7.4 compared to pH = 4 most likely reflects the protonation state of the host. The pK_a values of the three guanidiniocarbonyl pyrrole

Table 1. Association Constants K_{ass} (M^{-1}) Determined for the Binding of Anionic Substrates by Host **1** in Aqueous Solvents

substrate	K_{ass} (M^{-1})	pH	method[a]
glucuronic acid (**2**)	480		NMR
	3240	6.0	UV
galacturonic acid (**3**)	1550		NMR
	6160	6.0	UV
glucose-1-phosphate (**4**)	25 610	4.0	UV
	12 940	7.4	UV
galactose-1-phosphate (**5**)	21 150	4.0	UV
	12 160	7.4	UV
mannose-1-phosphate (**6**)	25 980	4.0	UV
	14 020	7.4	UV
methyl phosphate (**7**)	12 460	4.0	UV
	4850	7.4	UV

[a] NMR titration: 30% water in DMSO, error estimated to be ±10%; UV titration at pH = 4 (4 mM acetate buffer in 10% DMSO in water, $[\text{host}]_0 = 20\ \mu$M) or at pH = 7.4 (10 mM bis-tris buffer in 70% DMSO in water, $[\text{host}]_0 = 25\ \mu$M), error estimated to be ± 20%.

(15) This conformer is, however, calculated (Amber* force field, water GB/SA solvation model) to be only 2.4 kJ/mol less stable than the ground state conformation of **3**.

cations in **1** are between 6 and 8. Hence, at pH = 7.4, host **1** is mainly present in the monoprotonated form. This monocation has an intrinsically weaker affinity toward anions compared to the fully protonated tris-cationic host at pH = 4 even if this might to some extent be counterbalanced by a higher binding affinity of the now present phosphate dianions. Even so, efficient binding at pH = 7.4 requires a significantly less polar solvent (30% water in DMSO).

In contrast to the two uronic acids, all sugar phosphates bind nearly equally well with a slightly less efficient complexation of galactose compared to glucose and mannose. However, all sugar complexes are more stable by a factor of approximately 3 compared to simple methyl phosphate **7**. These trends are again reflected by the calculated complex structures (Figure 4). The phosphate group is bound by two

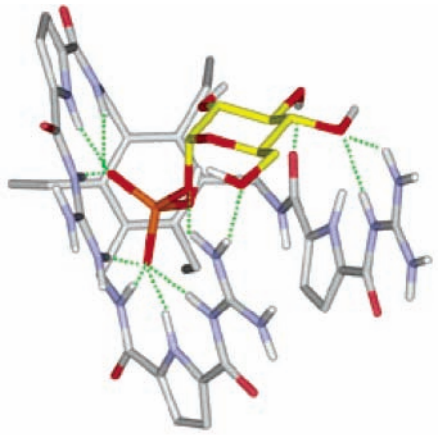

Figure 4. Calculated complex structure between **1** (gray) and **4** (yellow) showing the ion pair formation with the phosphate (left) and the additional H-bonds to the sugar OHs at C3, C4, and C6 (right). Nonpolar hydrogens have been omitted for clarity.

of the three cationic receptor arms. The two guanidinium groups each form a bidentate ion pair with two of the three negatively charged oxygens as expected for oxoanion binding by such guanidinium cations.[16] The third receptor arm interacts with the OH groups on C3, C4, and C6, forming both neutral (C3) and ionic hydrogen bonds (C4 and C6), respectively. Similar ionic H-bonds from a guanidinium cation to OH groups of monosaccharides are also found in Nature; e.g., in carbohydrate binding proteins, very often arginine plays an important role in sugar binding.[17]

The calculated structures also offer an explanation for the weaker binding of galactose phosphate **6** compared to either glucose (**4**) or mannose phosphate (**5**): The OH at C2 is not involved in interactions with the receptor. Hence, there should be no difference between mannose and glucose. However, the OH group at C4 is hydrogen-bonded by the third guanidinium group (Figure 4). Changing the configuration at this carbon from an equatorial to an axial OH group as in galactose is therefore expected to affect complex stability as experimentally observed.

The additional H-bonds to the sugar OH groups also explain the stronger binding of the anionic sugars compared to simple acetate or methyl phosphate. As complex formation takes place in water, each of these additional interactions is very weak, especially as the binding sites are quite open and therefore still well accessible by the solvent. However, in total, these interactions in addition to the ion pairing lead to a noticeable increase in complex stability.

In conclusion, we have shown here that binding of anionic sugars based on purely noncovalent electrostatic interactions (ion pair formation and H-bonds) in aqueous solvents is possible. A further optimization of the binding pocket and the recognition motifs in the sidearms, for example, using a combinatorial approach can now be used to improve both affinity and selectivity. Such work is currently in progress.

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Supporting Information Available: NOESY spectrum of the complex between **1** and **3** and binding data. This material is available free of charge via the Internet at http://pubs.acs.org.

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