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SUGAR BINDING TO AN CALIX[4]ARENE-BASED RECEPTOR

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Abstract – The binding properties of the calix[4]arene-based artificial receptor (**1**) toward carboxylate-containing sugars in DMSO are demonstrated. The receptor shows the 1:1 host-guest complexation with the sugars. The hydrogen bonds forming to the hydroxyl groups play a crucial role for the guest selection.

In nature saccharides are commonly found both at cell surface and in biological fluids.[1] Their structural features play a key role in biological events such as information transfer, intercellular communication, and molecular and cellular targeting.[2,3] In the early stage of the biological events, sugar-binding proteins find the key structure of a saccharide on a cell surface, and then bind it with hydrogen bonds even in water. Many carboxylate-containing sugars, such as neuramic acid, muramic acid, glucuronic acid and galacturonic acid etc, are known to play an important role in the biological events. The carboxylate groups can be key functionalities for effective binding of the carboxylate-containing sugars.

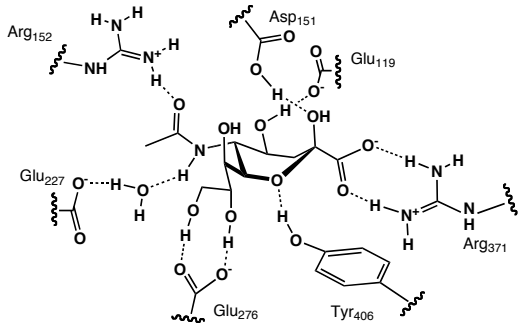

Figure 1. Schematic drawing of the interactions between residues of neuraminidase and sialic acid.

Neuraminidase is one of the well-known enzymes found on the surface of the Influenza virus, and is involved in the aid in virus release from infected cells. The enzyme recognizes and cleaves the terminal carboxylic acid residue from carbohydrate moieties of sialic acid on the surfaces of infected cells, promoting the propagation of virus. In this recognition event, the enzyme employs many charged residues to take up sialic acid with the hydrogen bonds into the binding pocket (Figure 1).[4] The carboxylate group of sialic acid is a key functionality for providing its specific binding inside the binding pocket. This characteristic hydrogen-bonded structure prompts us to elaborate an artificial synthetic receptor for the sugars. There have been many successful reports on the synthetic sugar receptors making use of the hydrogen bonds with the charged functionalites in lipophilic[5] and in polar[6] solvents; however, since limited examples on the selective recognition for carboxylate-containing sugars in polar solvents have been reported, [7] it is a challenging issue to develop a receptor, selectively binding to the carboxylate-containing sugars in a competitive solvent.

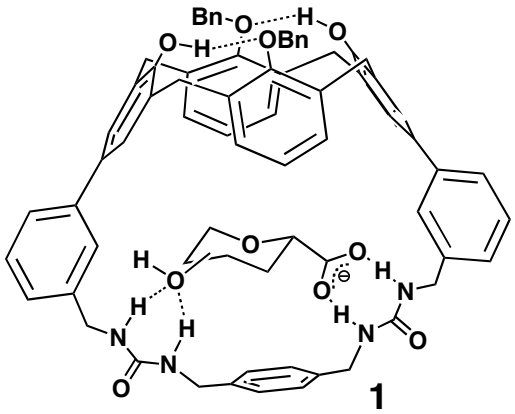

Figure 2. Schematic representation of sugar complexation with calix[4]arene-based receptor (**1**).

We have reported calixarene-based synthetic receptor (**1**) capable of binding neutral guests in organic medium.[8] In this paper, the selective binding of carboxylate-containing sugars (**2**-**8**) to calix[4]arene-based synthetic receptor (**1**)[9] is described (Figure 3). The two urea groups of **1** provide the pre-organized hydrogen-bonding environment with which the sugars can be taken up by the hydrogen bonds (Figure 2).[10] Binding of carboxylate-containing sugars to receptor (**1**) was studied by using ^{1}H-NMR titration technique in DMSO. When **2** was added to the solution of **1**, two N-H resonances attributed to the urea groups shifted downfield (Figure 4). This indicative shift supports the formation of the host-guest complex through hydrogen bonding interaction even in DMSO. To determine the stoichiometry of the complex, Job's plot was carried out. The Job's plot indicated the formation of the 1:1 complex between **1** and **2** (Figure 5). Non-linear least square curve fitting analysis[11] yielded an association constant, 200±10 M^{-1}.

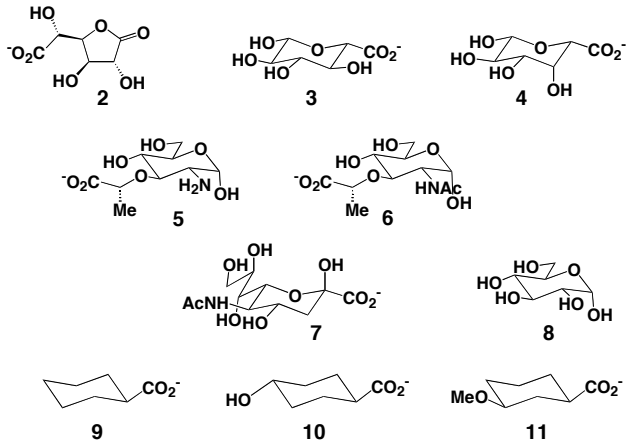

Figure 3. Sugars (**2**–**11**). Tetrabutylammonium is used as a counter cation for all carboxylates.

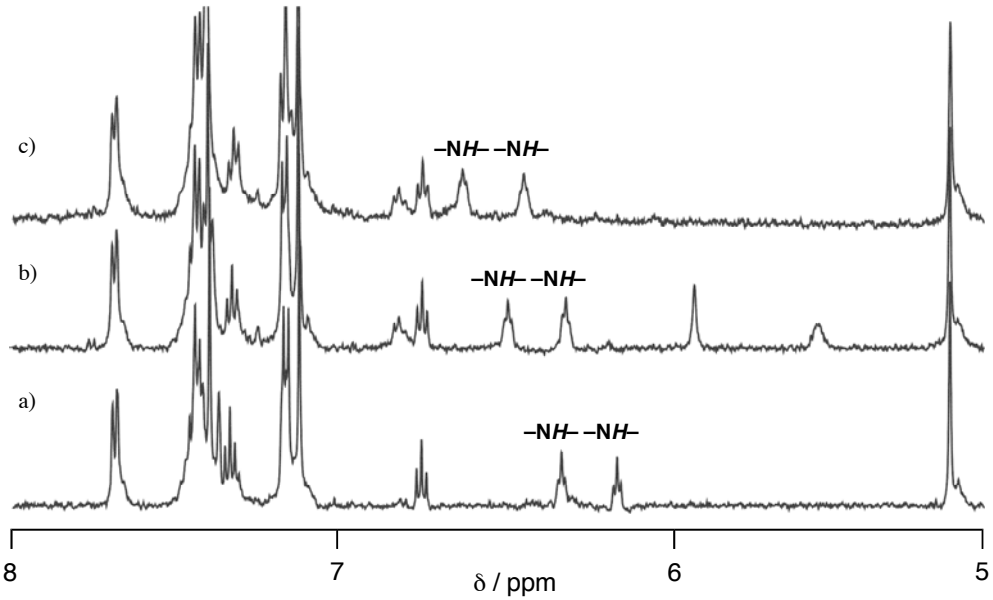

Figure 4. ^{1}H NMR spectra of a) **1** (1 X 10^{-3} mol/L), b) **1** and 0.2 eq. of **2**, c) **1** and 0.2 eq. of **3** in DMSO.

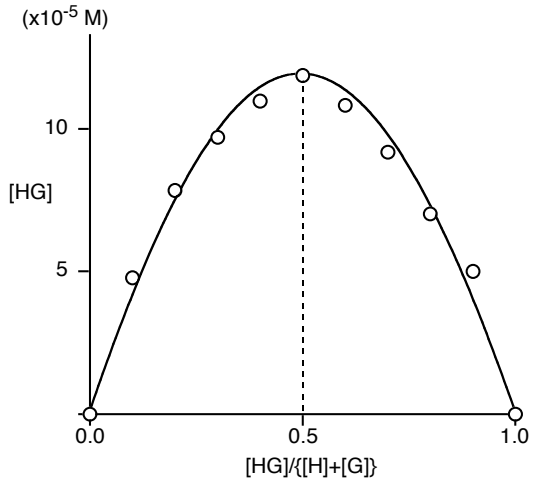

Figure 5. Job's plot of **1** and **2**.

Sugars (**3**-**8**) were used to evaluate their binding by **1** in DMSO. For each pair, the complexation was signaled by the same general pattern of the changes on the urea N-H resonances in ^{1}H-NMR spectroscopy as has been observed for **2**. Titration experiments for the sugars gave the respective binding constants (Table 1). Sugars having the carboxylate, (**2**-**7**), showed the effective binding to **1** while that without the functionality, **8**, did not. It indicated that the hydrogen bonding interaction between the urea and the carboxylate function play a key role in the production of the highly selective complexation. **1** recognized a tiny structural difference between sugar (**3**) and its epimer (**4**). More interestingly, the presence of basic amino group at the C-2 carbon of **5** and **6** does not interfere the formation of their hydrogen–bonded complexes with **1**. The steric bulkiness of the side chain of **7** decreased its binding affinity to **1**. These examples serve to illustrate how subtle factors; perhaps related to the additional hydrogen bonds forming the other urea groups can alter the recognition process.

Table 1. Binding constants (M^{-1}) for the receptor (**1**) with sugar guests at 293K in DMSO-d_6.

Sugar	K_a	Sugar	K_a
2	200 ± 10	**7**	91 ± 5
3	208 ± 8	**8**	0
4	115 ± 7	**9**	54 ± 5
5	200 ± 20	**10**	110 ± 10
6	190 ± 20	**11**	380 ± 30

In order to evaluate the contribution of the oxygen functionalities for the complexation, the titrations of cyclohexane carboxylates (**9**-**11**) were carried out. The affinity increased in the order of **9** to **11** implies that the oxygen functionalities provide some favorable contribution for the complexation. These results clearly indicate that the oxygen functionality at the C-3 carbon of six-membered ring provides the favorable cooperative interaction to the urea group. The formation of the hydrogen bonds between the urea and the hydroxyl group was confirmed by ^{1}H-NMR titration of **10** with **1**. The down field shift (0.21 ppm) of the hydroxyl proton of **10** was observed when an equivalent amount of **1** was added. This down field shift of the hydroxyl proton gives a proof that the hydrogen bonds are formed between the hydroxyl and urea groups.

The experimental results help us to propose the structures of complexes, **1** with the sugars, although the precise structures remain uncertain. Molecular modeling is helpful for understanding how the sugars situate in the binding environment provided with the two urea groups. Molecular mechanics calculations of complexes (**1**) and (**3**) or (**4**) were carried out using MacroModel V6.5 with MMFF force field.[12] The calculations gave us critical information on the guest selectivity. The calculated structures of the

complexes are shown in Figure 6. There are four hydrogen bonds (two urea N-H·····O-C(3) and two urea N-H·····O^--C=O) seen in the structures. The hydrogen bonds formed between the hydroxyl groups and the urea have sugar (**3**) situated on the lower aromatic ring of the receptor. Two axial hydrogen atoms on **3** are positioned on the lower aromatic ring, forming CH/π interaction. In the calculated structure with **4**, the axial hydroxyl group at the C-4 carbon, facing down to the lower aromatic ring, produces the steric repulsion, which forces sugar (**4**) not to stack on the lower aromatic ring. This steric interaction should be the key factor controlling the recognition selectivity seen in **3** vs. **4**.

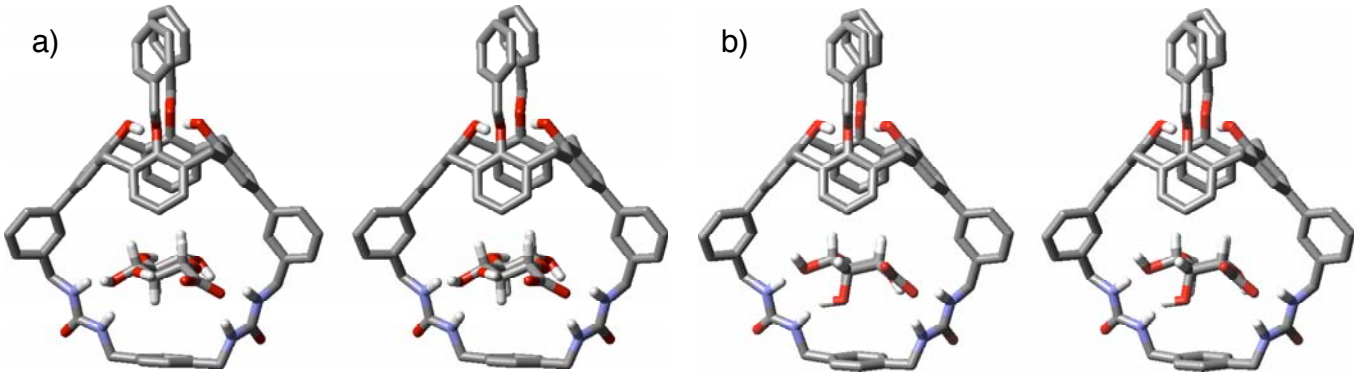

Figure 6. Stereo plots of the calculated complex structures of **1** (a) with **3** and (b) with **4**.

In summary, we demonstrated that the rationally arranged hydrogen bonding groups on the calix[4]arene can produce the effective gain of the cooperative hydrogen bonding to sugars even in the polar solvent. The receptor recognizes a tiny structural difference, which probably comes from the steric interaction of the bound sugars and the lower xylylene linker.

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