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THE MOLECULAR GEOMETRIES OF CYCLOFRUCTINS[1]

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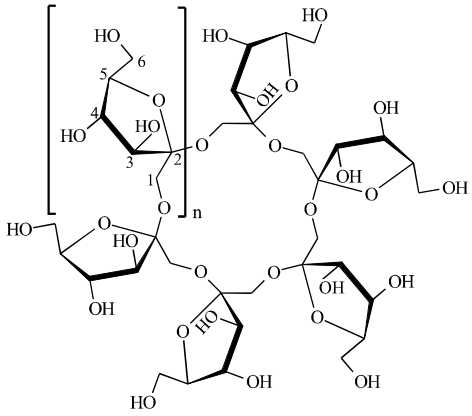
Abstract. Cyclofructins composed of six (CF_6, **1**) to ten (CF_{10}, **5**) β(1→2)-linked fructofuranose units (i.e. *cyclo*[D-Fru*f* β(1→2)]$_n$ with n = 6 - 10) were subjected to a detailed molecular modeling study. **1**, **2**, and **3** are disk-type shaped molecules without a cavity, whereas the nonamer (**4**) and decamer (**5**) adopt torus-like conformations that allow the formation of inclusion complexes similar to those of cyclodextrins.

1. Introduction

The cyclofructins[2] are a class of cyclooligosaccharides produced by the action of an extracellular enzyme of *Bacillus circulans* on the polysaccharide inulin; they are composed of six (**1**, CF_6), seven (**2**, CF_7), and eight (**3**, CF_8) β(1→2)-linked fructofuranose residues[3]. Although ring homologs with nine (**4**, CF_9) and ten (**5**, CF_{10}) fructose residues have as yet not been uncovered in enzymatic digests of inulin, the unspecificity of the *Bacillus circulans*-derived enzyme may lead to their formation and eventual isolation.



cyclofructins

1	*cyclo*[D-Fru*f* β(1→2)]$_6$	(CF_6)
2	*cyclo*[D-Fru*f* β(1→2)]$_7$	(CF_7)
3	*cyclo*[D-Fru*f* β(1→2)]$_8$	(CF_8)
4	*cyclo*[D-Fru*f* β(1→2)]$_9$	(CF_9)
5	*cyclo*[D-Fru*f* β(1→2)]$_{10}$	(CF_{10})

Experimental[4] and theoretical[5,6] studies have established the ability of the cyclofructins **1 - 3** to complex cations through electrostatic interactions with their characteristic crown ether skeletons. The question whether the cyclofructins of different ring sizes are able to form inclusion complexes similar to those formed by cyclodextrins[7] initiated our molecular modeling studies on **1 - 5** to be described in the sequel.

2. Methods

From the solid-state geometry of CF_6 trihydrate[8] (**1**) two different fructofuranosyl geometries of the asymmetric unit were assembled to symmetrical and asymmetrical macrocycles **1** - **5**, and for each of these starting geometries unrestricted "*Monte-Carlo*" (MC) and "*random-walk*" simulations[9] using adapted corner-flapping procedures[10] were initiated (PIMM91 force-field[11]). Out of the total of approx. 130 000 structures generated for each compound, the global energy-minimum structures[12] were used for the surface calculations and the molecular modelings utilizing the MOLCAD program[13].

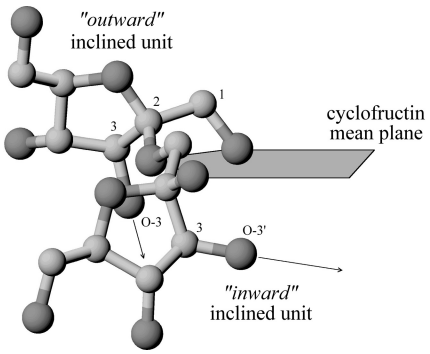
MD simulations of the CFs in aqueous solution were carried out with the CHARMM[14] force-field[15] (timestep 1 fs, total simulation time ca. 750 ps for each CF) using periodic boundary conditions of the truncated octahedron (box size 32 Å, boxes filled with 606 - 558 water molecules for **1** - **5**).

3. Results and Discussion

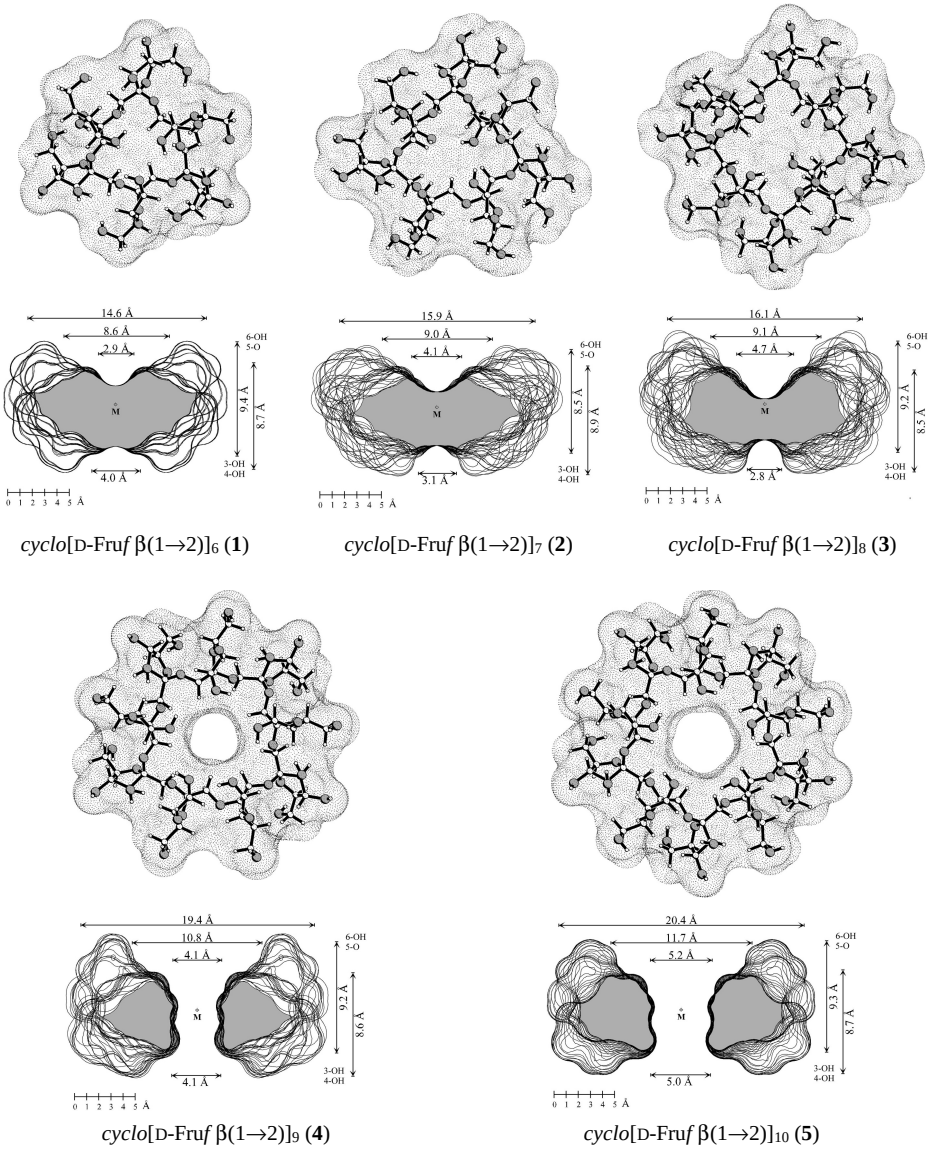
Conformations of the Cyclofructins. — The global energy-minimum structures of the cyclofructins **1** - **5** obtained from Monte-Carlo and molecular dynamics simulations (vide supra) are shown in Fig. 1[12] together with their dotted contact surfaces[16] and the corresponding cross-section plots. Here, only the global shapes of the macrocycles are discussed; a detailed description of all used and calculated molecular geometry parameters will be published elsewhere.

The fructofuranosyl residues bear some degree of flexibility, preferring $^4T_3 \rightleftharpoons E_3$ conformations with medium ring distortions. They are linked in a rigid, *spiro*-type fashion to the crown ether backbone of the macrocycles, which exhibit nearly perfectly planar *n*-polygons of the O-1 atoms. The primary 6-CH_2OH groups and the furanoid ring oxygens O-5 are located on one side of the molecule, and the secondary 3-OH and 4-OH groups on the other.

The most significant feature of the cyclofructins turned out to be a high preference for an alternating arrangement of the fructofuranoses being inclined "*inward*" (down) and "*outward*" (up) with respect to the mean plane of the macrocyclic backbone. This entails that half of the 3-OH groups point towards the center of the macrocycle ("*inward*" rotation), whereas the others are situated nearly perpendicular on the ring perimeter ("*outward*" tilting).



The even-membered cyclofructins **1**, **3**, and **5** prefer $C_{n/2}$ symmetrical structures, whereas for CF_7 (**2**) and CF_9 (**4**) the alternating alignment of the odd number of furanose residues is retained as largely as possible, but results in C_1 symmetry. Most notably, the computed structure for CF_6 (**1**) matches almost perfectly the geometry realized in the solid-state[8].



cyclo[D-Fru*f* β(1→2)]$_6$ (**1**) *cyclo*[D-Fru*f* β(1→2)]$_7$ (**2**) *cyclo*[D-Fru*f* β(1→2)]$_8$ (**3**)

cyclo[D-Fru*f* β(1→2)]$_9$ (**4**) *cyclo*[D-Fru*f* β(1→2)]$_{10}$ (**5**)

Figure 1. Ball-and-stick model representations of the global energy-minimum structures of the cyclofructins **1** - **5** with the contact surfaces superimposed in dotted form. Structures are shown perpendicular to the mean ring plane of the macrocycles; the 3-OH / 4-OH side of the furanoid rings points towards the viewer, and the 6-CH_2OH groups and the 5-O atoms away from him; oxygens are shaded. Surface cross-section contours (lower entries each) were obtained for successive 10° rotation steps around the geometrical center *M* and superimposed. In each case the 6-CH_2OH groups are located on the top, and the 3-OH / 4-OH groups on the bottom side of the cross-cut models; approximate molecular dimensions are given in Å.

In addition to the force-field calculations on the isolated molecules (vacuum conditions), molecular dynamics simulations on **1** - **5** (cf. Fig. 2) in aqueous solution provided evidence for the relevance of these conformations in water.

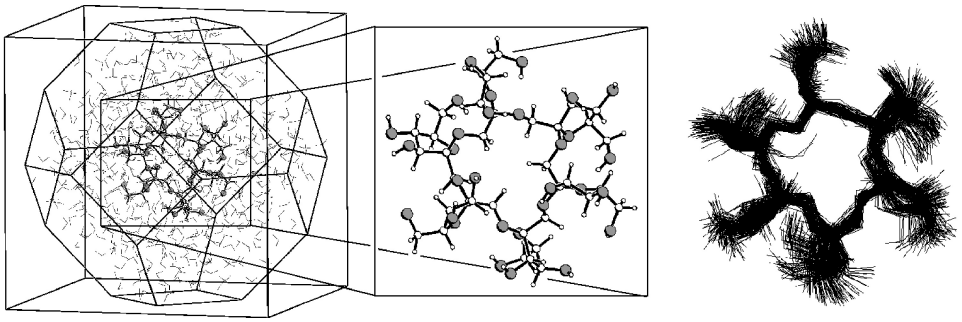
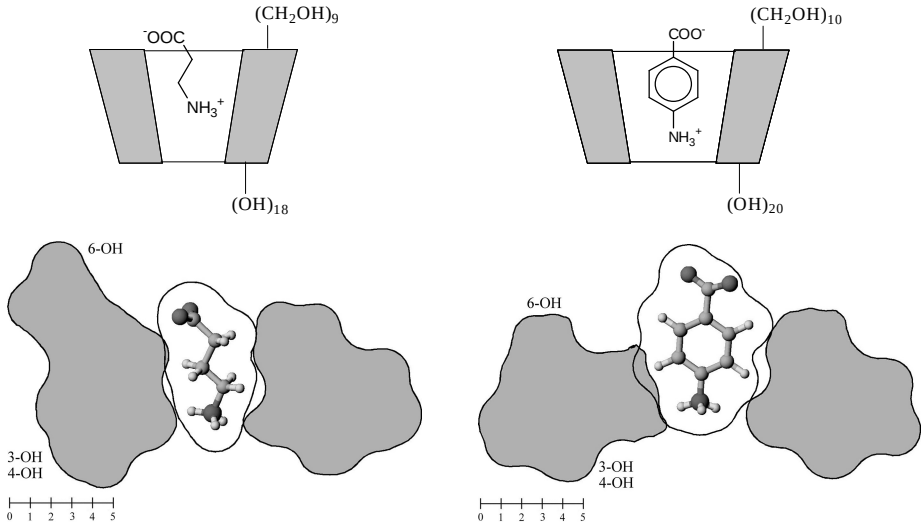


Figure 2. Truncated octahedron MD-simulation box of CF_6 (**1**) filled with 606 water molecules (left). In the middle, an exemplary solute conformation is zoomed out; on the right side, 100 solute snapshot geometries taken from a 750 ps trajectory are fitted and superimposed. In solution, the over-all shape of the vacuum energy-minimum conformation is largely retained.

The atomic distances between the O-1 crown ether oxygens diagonally across the macroring increase gradually from approximately 6.3 Å for CF_6 (**1**) to 10.2 Å in CF_{10} (**5**). In a more transparent way, the MOLCAD[13] program-generated contact surfaces[16] and the corresponding cross-section cuts[17] (cf. Fig. 1) display the effective shapes and molecular extensions of the CFs (approx. distances given in Fig. 1). In contrast to the nearly perfectly round-shaped cyclodextrins, the alternating "*inward / outward*" inclination of the fructofuranosyl residues renders the surface cuts of **1** - **5** with a deeply fissured, irregular over-all shape. Clearly, **1**, **2**, and **3** feature disk-type shapes with central indentations only, whereas **4** and **5** have torus-like (doughnut) appearances and "through-going" round-shaped cavities with diameters of approx. 4.1 Å and 5.2 Å, respectively.

Inclusion Complexation Potential of CF_9 and CF_{10}. — On the basis of the steric requirements for the formation of inclusion complexes, it is evident that CF_9 (**4**) is able to accommodate linear alkyl chains in *all-trans* zigzag conformations, whereas the CF_{10} (**5**) cavity is wide enough to fit aromatic rings in a cyclodextrin-like fashion. As typical examples, the mode of incorporation of β-alanin and *p*-aminobenzoic acid into the cavities of **4** and **5** is visualized in Fig. 3 by the corresponding surface cross-section cuts for the computer-generated global energy-minimum structures. Of the two possible guest-host alignments, the one shown in Fig. 3 is highly favored: in both complexes **6** and **7** the carboxylate groups of the guest molecules are located close to the 6-CH_2OH side of the macrocycles, whereas the amino functions are situated at the opposite side close to the 3-OH and 4-OH groups.



CF_9 · β-alanin (**6**) CF_{10} · p-aminobenzoic acid (**7**)

Figure 3. Surface cross section cuts through the global energy-minimum structures of the inclusion complex of CF_9 with β-aminopropionic acid (**6**, left) and the CF_{10} *p*-aminobenzoic acid adduct (**7**, right). The geometries depicted correspond to the energetically "*matched*" host-guest orientations; in both cases the host molecule was omitted for clarity.

Further insights into the guest complexation capabilities of the cyclofructins **4** and **5** are expected to emerge from generation of their molecular electrostatic potential profiles and lipophilicity patterns, which are surmised to provide a reliable assessment of the electrostatic interactions between the guest and host, as well as an indication to which extent hydrophobic effects contribute to the stability of the inclusion complexes; studies along this vein will be published in due course.

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