# Cyclofructins: Conformations, Molecular Electrostatic and Lipophilicity Patterns, and Inclusion Complexes

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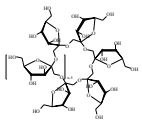
## Introduction

The cyclofructins[1] 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[2]. 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. Experimental[3] and theoretical[4,5] 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[6] initiated our molecular modeling studies on **1** - **5** to be described here.



**Cyclofructins**

| | | |
|---|---|---|
| 1 | $n = 6$ | *cyclo*[D-Fruf β(1→2)]$_6$ |
| 2 | $n = 7$ | *cyclo*[D-Fruf β(1→2)]$_7$ |
| 3 | $n = 8$ | *cyclo*[D-Fruf β(1→2)]$_8$ |
| 4 | $n = 9$ | *cyclo*[D-Fruf β(1→2)]$_9$ |
| 5 | $n = 10$ | *cyclo*[D-Fruf β(1→2)]$_{10}$ |

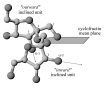
From the solid-state geometry of $CF_6$ trihydrate[7] (**1**) two different fructofuranosyl geometries of the asymmetric unit were assembled to symmetrical and asymmetrical macrocycles **1** - **5**, and for each of these starting geometries unrestricted "*Monte-Carlo*" (MC) and "*random-walk*" simulations[8] using adapted corner-flapping procedures[9] were initiated (PIMM91 force-field[10]). Out of the total of approx. 130000 structures generated for each compound, the global energy-minimum structures[11] were used for the surface calculations and the molecular modelings utilizing the MOLCAD program[12]. MD simulations of the CFs in aqueous solution were carried out with the CHARMM[13] force-field[14] (timestep 1fs, total simulation time ca. 750 ps for each CF) using periodic boundary conditions of the truncated octahedron (box size 32Å, boxes filled with 606 - 558 water molecules for **1** - **5**).

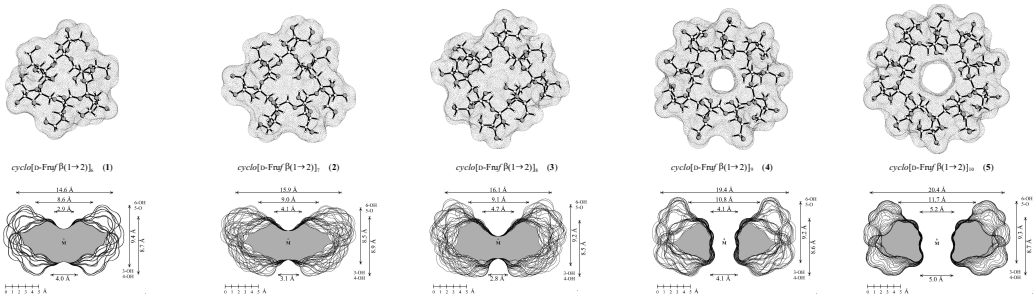
## Molecular Geometries of the Cyclofructins

The global energy-minimum structures of the cyclofructins **1** - **5** obtained from Monte-Carlo and molecular dynamics simulations (vide supra) are shown in below[11] together with their dotted contact surfaces[11] and the corresponding cross-section plots. The fructofuranosyl residues bear some degree of flexibility, preferring $^{T}E \leftrightarrow E_{2}$ 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 even-membered cyclofructins **1**, **3**, and **5** prefer $C_{nv}$ 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. In a more transparent way, the MOLCAD[12] program-generated contact surfaces[11] and the cross-section cuts[11] display the effective shapes of the CFs. 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 appearances and "through-going" round-shaped cavities.

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).



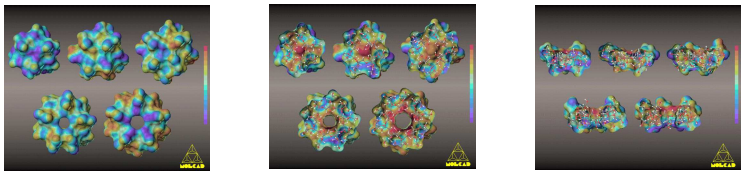



*cyclo*[D-Fruf β(1→2)]$_6$ (**1**) — *cyclo*[D-Fruf β(1→2)]$_7$ (**2**) — *cyclo*[D-Fruf β(1→2)]$_8$ (**3**) — *cyclo*[D-Fruf β(1→2)]$_9$ (**4**) — *cyclo*[D-Fruf β(1→2)]$_{10}$ (**5**)

## Molecular Electrostatic and Lipophilicity Patterns

### Molecular Electrostatic Profiles

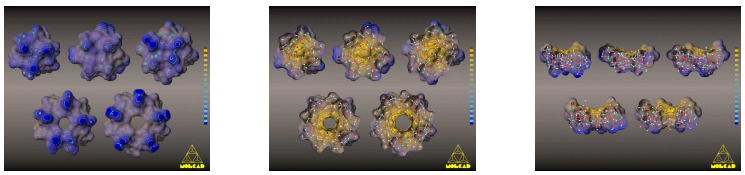
Molecular electrostatic potential profiles (MEPs) on the contact surfaces of the β-(1→2)-linked cyclofructins **1** - **5** with six (**1**, upper left), seven (**2**, upper middle), eight (**3**, upper right), nine (**4**, lower left), and ten (**5**, lower right) fructose units. The 16 color code ranging from violet (most negative potential) to red (most positive potential) is applied in relative terms for each molecule separately; the molecular orientations and the modes of viewing correspond to the dotted surface models shown above.



View: 3,4-OH →1,6-$CH_2$ — View: 3,4-OH →1,6-$CH_2$ (open) — Side view: 6-$CH_2$OH up, 3,4-OH down (open)

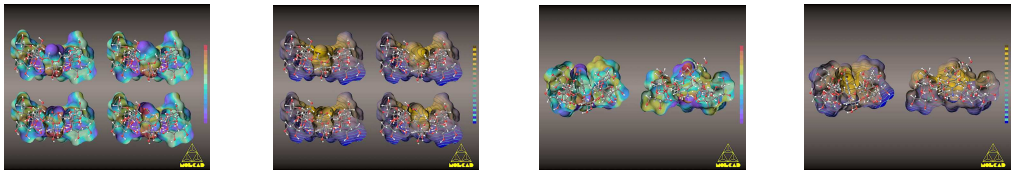
### Molecular Lipophilicity Patterns

MOLCAD program generated MLPs[12,16] projected onto the contact surfaces of $CF_6$ (**1**, upper left), $CF_7$ (**2**, upper middle), $CF_8$ (**3**, upper right), $CF_9$ (**4**, lower left), and $CF_{10}$ (**5**, lower right). For visualization a two-color code graded into 32 shades is used. The color coding is adapted to the range of relative hydrophobicity calculated for each molecule, by using 16 colors ranging from dark blue (most hydrophilic areas) to full yellow (most hydrophobic regions). The remaining 16 color shades (light blue to brown) are used to indicate iso-contour lines in between former color scale. The left picture exposes the intensively hydrophilic (blue) 3-OH/4-OH side of the molecules. In the center, the front half of the surfaces has been removed, providing an inside view onto the hydrophobic (yellow) backsides. The bisected side-view MLPs (right) allow an inside view on the hydrophobic central cavities of **4** and **5**.



## Inclusion Complexes

As typical examples the energy-minimum structures of the inclusion complexes β-alanine · $CF_6$, γ-aminobutyric acid · $CF_6$, and *p*-aminobenzoic acid · $CF_{10}$ are shown below. Of the two possible guest-host alignments, the one shown is highly favored due to the antiparallel arrangement of dipoles (cf. MEPs, "*matched*" pair). The MLPs visualize the mode with which the hydrophobic parts of the guests molecules are incorporated into the cyclofructin cavities.



$CF_6$(**4**) with β-aminopropionic acid — $CF_6$(**4**) with γ-aminobutyric acid — $CF_7$(**4**) with β-aminopropionic acid — $CF_7$(**4**) with γ-aminobutyric acid — $CF_{10}$(**5**) with *p*-aminobenzoic acid — $CF_{10}$(**5**) with *p*-aminobenzoic acid

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