# Flexible and Rigid Non-glucose Cyclooligosaccharides: Synthesis, Structure, and Properties

*Habilitationsschrift*

Dem Fachbereich Chemie der Technischen Universität Darmstadt
für das Fachgebiet Organische Chemie vorgelegt von

**Stefan Immel**

Darmstadt, 2003

## Annotations

This thesis is divided into nine individual Chapters providing the original publications related to specific aspects of this work; all references are given within these publications. A short general introduction and a synopsis of the results have been added before the first Chapter.


## Acknowledgments

This work has been conducted at the Clemens-Schöpf-Institut für Organische Chemie und Biochemie, Technische Universität Darmstadt, and is the result of sharing of ideas addressed by a number individuals over the past several years. Acknowledging specific individuals by name carries the risk of overlooking key contributors: however, it would be a gross oversight not to recognize at least a few individuals for their support, unwavering encouragement, advocacy, and many lucid and critical discussions and suggestions. Above all, I would like to acknowledge my mentor Prof. Dr. F. W. Lichtenthaler, as well as Prof. Dr. J. Brickmann and Prof. Dr. H. J. Lindner for their valuable advice and support. Highly appreciated are also the contributions that have emerged from international cooperations with Prof. Dr. T. Nakagawa and Prof. Dr. K. Fujita. Not mentioned individually, but not less valuable, I would like to thank all my coauthors of my publications for having contributed to this work.

November 2003 STEFAN IMMEL

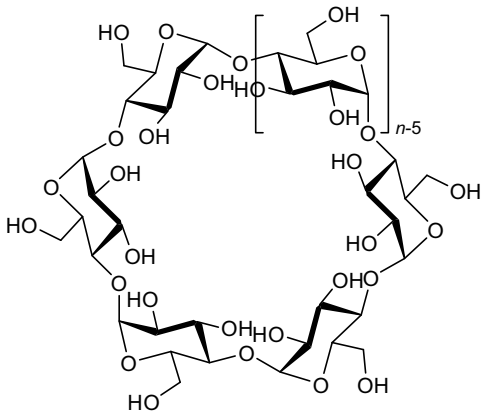
## Table of Contents

# Flexible and Rigid Non-glucose Cyclooligosaccharides: Synthesis, Structure, and Properties

## Introduction

At present, the evaluation of molecular recognition processes represents one of the most challenging fields of research. Molecular interactions are vital to almost every phenomenon in the field of chemistry, biochemistry, and biology, and many of the fundamental principles still remain to be fully understood. Amongst these processes, receptor-substrate and host-guest recognition are of particular importance. The amylose-derived natural cyclodextrins (CDs), small cyclo-oligosaccharides made up out of six or more α(1→4)-linked D-glucose residues, represent a prominent class of potential hosts to a wide range of small organic molecules. Their unique ability to include hydrophobic guest molecules into their hydrophobic central cavities in aqueous solution has been studied extensively and has led to numerous commercial applications in the field of e.g. pharmaceutics, cosmetics, drug stabilization and delivery, biotechnology, and environmental applications. Among the driving forces for the formation of these inclusion complexes are dipole-dipole interactions and - most notably - hydrophobic interactions caused by the H-3 and H-5 protons of the glucose units pointing towards the center of the cyclodextrin cavities.

The molecular geometry of the cyclodextrins closely resembles truncated cone structures with the primary 6-$CH_2OH$ and the secondary 2- and 3-OH groups of the glucose moieties lined up on opposite sides of the macrocyclic torus. As evidenced by a large number of X-ray or neutron diffraction derived solid-state structures of α-, β-, and γ-cyclodextrins and their inclusion complexes, these host molecules adopt rather rigid conformations with respect to the almost perpendicular alignment of the glucose residues in relation to the mean-planes of the macrocycles. In general, only little or no conformational changes are observed within the hosts upon inclusion of potential guest



**cyclodextrins**

*cyclo*[D-Glc*p* α(1→4)]$_n$

**α-cyclodextrin** (*n* = 6)
**β-cyclodextrin** (*n* = 7)
**γ-cyclodextrin** (*n* = 8)

molecules. Further evidence for the greatly limited flexibility of the cyclodextrins stems from numerous NMR and theoretical (molecular mechanics and dynamics) studies on these assemblies. If at all, only slight elliptical distortions to accommodate elongated or disc-shaped (aromatic) guests are observed in the cyclodextrin complexes. With very few exceptions, the energetically favorable rigid $^4C_1$ chair geometries of the glucose units are retained in all structures reported.

The molecular recognition abilities of the native cyclodextrins are greatly confined by their rigid $C_n$ symmetrical structures, and, in general, the formation of inclusion complexes displays a significant degree of shape selectivity as the hosts are incapable of undergoing major conformational changes to adopt their shape towards guest molecules. This is of particular importance, as cyclodextrins have been treated as model structures for biomimetic recognition processes and enzyme models in the context of catalysis. However, when comparing the cyclodextrins with highly flexible structures such as enzymes a fundamental discrepancy becomes obvious: whereas enzymes recognize their substrates through conformational changes – which finally trigger the biological response – in an flexible *induced-fit*-type manner, the formation of inclusion complexes by cyclodextrins represents a rather rigid *lock-and-key*-type process. In general, cyclodextrins display little – if any – tendency to undergo significant conformational changes or to adapt their shape towards guest molecules. Therefore, in catalytic processes, cyclodextrins are rigid templates rather than flexible enzyme models, exerting their catalytic activity by bringing reactive groups and substrates in close proximity in the inclusion complexes. Chapter 1 of this work describes the molecular geometry of the yet unknown inclusion complex of $\alpha$-cyclodextrin with nitromethane. In addition, the structures and conformational properties of cyclodextrins with increasing ring size are compared to the geometry of amylose, pointing out significant differences and similarities.

In view of the obvious differences in molecular recognition by enzymes and cyclodextrins, it was of fundamental importance to develop flexible cyclodextrin hosts and study their inclusion complexes to obtain more realistic enzyme models. In this work, efforts directed towards the efficient synthesis of flexible cyclodextrin-derived molecular hosts are described along with their unequivocal structural and geometrical characterization in the solid-state, and in solution. In principle, the following approaches are appropriate to introduce conformational flexibility into the cyclodextrins:

- Per-*O*-alkylation or per-*O*-acylation introduces steric hindrance between the glucose residues, leading to more distorted structures of higher flexibility with increased ranges of accessible tilt angles between the constituent sugars. Simultaneously, the torus stabilization through intramolecular hydrogen bonds is reduced, or even totally eliminated.

- Deoxygenation of hydroxyl groups introduces flexibility through disruption of intramolecular hydrogen bonds (→ per-2,3-anhydro-α-cyclomannin, Chapter 2).
- Transformation of the rigid glucose moieties into more flexible sugar residues such as α-D-altrose (→ cycloaltrins and mono-*altro*-cyclodextrins, Chapter 3 and 4); or replacement of the pyranose rings by more flexible furanoid structures (→ cyclofructins and cyclogalactofuranosides, Chapter 5).
- Cleavage of C-C-bonds in the constituent pyranoses generates highly flexible macrocycles (→ crown acetals, Chapter 6).

In all cases listed above, the flexibility of the new cyclodextrin-derived hosts is expected to drive the corresponding mechanism of inclusion complexation towards a more flexible *induced-fit*-type process of molecular recognition. However, it is also important to study the effects of the opposite approach, i.e. introducing enhanced rigidity into the hosts. Within this work, two routes towards rigidified cyclodextrin derivatives are pursued (Chapter 7):

- Intramolecular bridging between the constituent saccharide units leading to anhydro-cyclodextrins.
- Intermolecular bridging and generation of cyclodextrin dimers with the focus on short, single atom linkages between the macrocycles.

For both approaches, examples are detailed in Chapter 7, and the molecular structures of new intra- and intermolecularly bridged cyclodextrin derivatives are outlined.

## Synopsis

In the sequel, brief outlines are provided for each of the Chapters in this work. All details and the corresponding references are given in the original publications included after this summary in Chapter 1 - 9.

### 1. From Cyclodextrins to Amylose: Structures and Lipophilicity Patterns

Although cyclodextrins have been crystallized from a wide range of organic solvents (e.g. methanol, dimethylformamide, and dimethyl sulfoxide) and were characterized by the solid-state structures of the corresponding solvate hydrates, nitromethane, a standard organic solvent as well as a reagent, has not been reported to complex with α-CD or any of the other cyclodextrins. In this context, suitable crystals for solid-state structural analysis were obtained by slow crystallization of α-CD from a 9:1 water–nitromethane solution. In the crystal structure of the α-CD – nitromethane pentahydrate complex, the comparatively unrestrained overall geometry of the α-CD host is stabilized through an almost complete ring of O-2···O-3

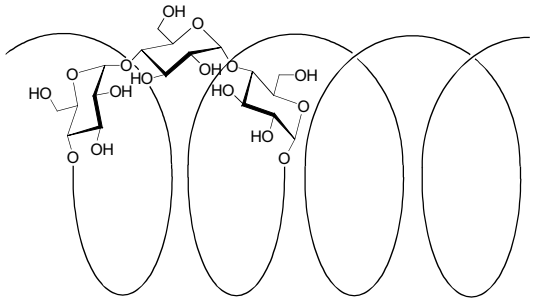
hydrogen bonds between five out of six adjacent glucose residues. The inclusion of nitromethane almost perpendicular to the molecular axis displays a counterbalance of hydrophobic effects and favorable antiparallel arrangement of dipoles, while the α-CD cavity itself is large enough to allow for excessive thermal motions of the guest and rotation of the nitro group. The ready preparation of the complex, and the subtleties of its molecular structure clearly demonstrate the ease with which water is expelled from the α-CD cavity by a co-solvent.

For details see Chapter 1, publication:

Topography of the 1:1 α-Cyclodextrin - Nitromethane Inclusion Complex.
T. Nakagawa, S. Immel, F. W. Lichtenthaler, and H. J. Lindner,
*Carbohydr. Res.* **2000**, *324*, 141-146.

A molecular modelling study on the A- and $V_H$-polymorphs of amylose based on fiber X-ray diffraction data was carried out, pointing out structural analogies to the cyclodextrins. Both amylose forms represent rod-shaped, left-handed helices of α(1→4)-linked D-glucose units. The A-form consists of parallel-stranded double helices with a pitch of approximately 21.4 Å; the structure of tightly twisted helices does not feature central cavities or channels that are accessible to the formation of inclusion complexes. On the other hand, the $V_H$-polymorph is made up of single helices with six glucose residues per turn and an average axial spacing of 8.05 Å per turn. In this respect, the structure of $V_H$-amylose strongly reminds that of α-cyclodextrin with an average torus height of 8.1 Å, for both structures the inner cavity or channel displays a diameter of 5.0 – 5.5 Å. $V_H$-amylose does indeed form inclusion complexes with extended guest molecules such as fatty acids or long chain alcohols. In this study, the structure of the well-known, dark deep-blue colored iodine / iodide complex of amylose was compared to that of the corresponding complex formed by α-cyclodextrin in the solid-state. Although both complexes are comparable in terms of cavity size and axial spacing, the alternating head-to-head and tail-to-tail arrangement of the α-cyclodextrin units along a central axis results in distinctly different patterns in the distribution of the hydrophobicity regions on the surfaces of the molecular assemblies.

**$V_H$-amylose**

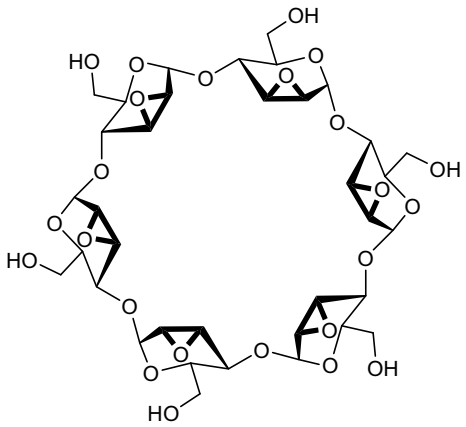


Based on molecular dynamics simulations in water, the structures of A-amylose are compared to structural motifs emerging in the solid-state structures of cyclodextrins with increasing ring size. Most notably, helical sub-structures with approximately six glucose units per turn seem to be the most predominant motif even in the geometries of the very large-ring cyclodextrins.

For details see Chapter 1, publication:

The Hydrophobic Topographies of Amylose and its Blue Iodine Complex.
S. Immel and F. W. Lichtenthaler,
*Starch/Stärke* **2000**, *52*, 1-8.

## 2. Per-2,3-anhydro-α-cyclomannin



**per-2,3-anhydro-α-cyclomannin**

The key-intermediate towards the synthesis of highly flexible cyclodextrin-derived artificial hosts turned out to be per-2,3-anhydro-α-cyclomannin. Chapter 2 details the straight forward synthesis of this compound starting from α-cyclodextrin in three convenient steps: protection of the 6-$CH_2OH$ groups by *tert*-butyl-dimethylsilyl moieties is followed by one-step treatment with NaH / DMF and subsequent benzenesulfonation. This not only yields the selectively 2-*O*-sulfonated α-cyclodextrin, but also concomitantly displaces the 2-sulfonyloxy groups by the vicinal 3-OH groups. Finally, deblocking of the primary hydroxyl groups yields the per-2,3-anhydro-α-cyclomannin in crystalline form and a total yield of about 48% over all steps. By precipitation from either aqueous ethanol or *n*-propanol, single crystals were obtained which allowed for an unequivocal structural characterization of this compound by X-ray crystal structure analysis. In each case, the corresponding ethanol or *n*-propanol complex was obtained in hydrated form. The macrocycles display an almost identical overall shape of approximate $C_6$ symmetry, resembling six-pointed stars with the epoxide rings being directed towards the outside of the cyclomannins, pointing away almost perpendicular from the central molecular axis. In both crystal structures, the arrangements of the macrocycles in an alternating head-to-head, tail-to-tail, and head-to-tail type fashion is almost identical. However, the mode with which the ethanol or *n*-propanol guest molecules are included differs significantly from each other: whereas two ethanol molecules form hydrogen-bonded dimers between two adjacent cavities of their hosts, the included *n*-propanol is hydrogen bonded to the 6-$CH_2OH$ of the cyclomannins, and its alkyl chain faces the hydrophobic chain of an adjacent *n*-propanol in the molecular stacks formed in the crystal lattice.

Most characteristically, by formation of the six anhydro (epoxide) rings from the altogether 12 secondary hydroxyl groups of α-cyclodextrin, the distribution of hydrophilic and hydrophobic surface regions on the outside of both hosts is entirely reversed. Although the torus rim carrying the primary 6-OH groups of α-cyclodextrin was less hydrophilic than the opposite rim made up by the secondary 2- and 3-OH groups, in the case of per-2,3-anhydro-α-cyclomannin this 6-$CH_2OH$ aperture is by far more hydrophilic; within this Chapter, consequences of this finding on the process of molecular recognition are discussed.

The synthesis of per-2,3-anhydro-α-cyclomannin not only provided the key-intermediate towards the efficient generation of highly flexible cyclodextrin-derived hosts – the cycloaltrins – but also for the first time the inclusion complexes of non-glucose cyclooligosaccharides have been obtained and unequivocally characterized by their solid-state structures.

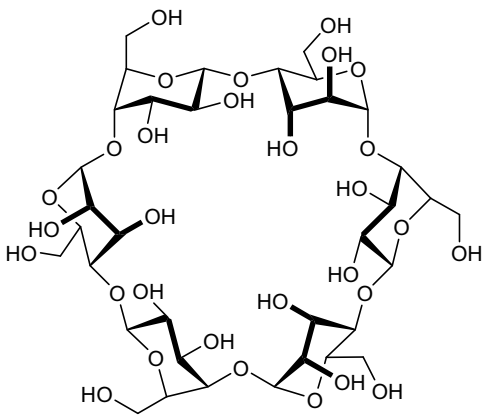
For details see Chapter 2, publications:

Structure and Lipophilicity Profile of 2,3-Anhydro-α-cyclomannin and its Ethanol Inclusion Complex.
S. Immel, K. Fujita, H. J. Lindner, Y. Nogami, and F. W. Lichtenthaler,
*Chem. Eur. J.* **2000**, *6*, 2327-2333.

The 2,3-Anhydro-α-cyclomannin - 1-Propanol Hexahydrate: Topography, Lipophilicity Pattern, and Solid-state Architecture.
S. Immel, F. W. Lichtenthaler, H. J. Lindner, K. Fujita, M. Fukudome, and Y. Nogami,
*Tetrahedron: Asymmetry* **2000**, *11*, 27-36.

### 3. α-Cycloaltrin

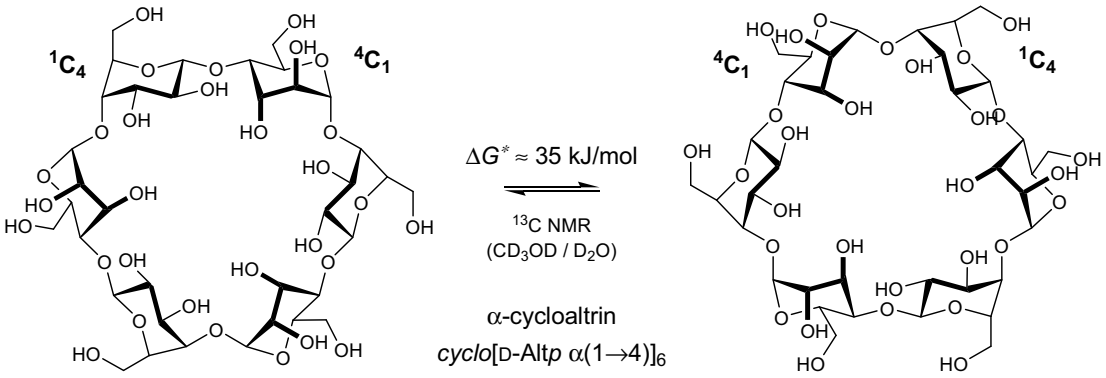
With the synthesis of per-2,3-anhydro-α-cyclomannin, the first thoroughly flexible non-glucose cyclooligosachharide α-cycloaltrin became accessible through nucleophilic ring opening of all epoxide rings by water. The axial 2- and 3-OH groups of the constituent α-D-altrose residues, as well as the axially disposed anomeric substituents leave these saccharide units highly flexible. Analysis of various solid-state structures of α-D-altrose derivatives, as well as detailed molecular dynamics studies and free-energy calculations on this sugar unit in aqueous solution, provided evidence for both $^4C_1$ and $^1C_4$ conformations of α-D-altrose having almost equal energies, and even intermediate $^OS_2$ skew-boat geometries become energetically accessible. Based on NMR investigations on α-cycloaltrin, neither of the above forms is realized exclusively, but surprisingly the chemically identical altrose residues adopt alternating arrangements of $^4C_1$ and $^1C_4$ structures in this macrocycle. Thus, the



**α-cycloaltrin**
*cyclo*[D-Alt*p* α(1→4)]$_6$

shape of α-cycloaltrin is reduced from $C_6$ to $C_3$ rotational symmetry only. The NMR data provided evidence for the intramolecular presence of multiple altrose conformers, but not for an equilibrium between structures of the all-$^4C_1$ and all-$^1C_4$ type. α-Cycloaltrin displays dynamic exchange between $[^4C_1 / {}^1C_4]_3$ and $[^1C_4 / {}^4C_1]_3$ forms in (aqueous) solution with an activation barrier of approximately 35 kJ/mol at room temperature.

Molecular dynamics simulations on α-cycloaltrin in solution, with additional constraints applied to certain torsion angles, shed some light on the mechanism of the process of conformational rearrangements: as one α-D-altrose unit is forced to interconvert from a $^4C_1$ into a $^1C_4$ geometry via intermediate $^OS_2 \leftrightarrow {}^{3,O}B$ forms, neighboring altrose residues engage in conformational transitions of the type $^1C_4 \rightarrow {}^OS_2$, too. Obviously, within the strait-jacket of the macrocyclic ring, the altrose can not be regarded separately, but conformational changes in any unit introduce rearrangements of the neighboring residues. In total, this process, once initiated, rolls around the macrocycle until finally all pyranose chair geometries are inverted. With this mechanism, the α-cycloaltrin represents the first example of a highly flexible cyclooligosaccharide, for which conformational transitions result in a pseudorotational type behavior: conformational exchange results in structures which simply seem to have emerged from a rotation of the initial geometry around its central axis. In this work, further new and unique examples on pseudorotational phenomena in cyclooligosaccharides are described (see Chapter 5, the cyclofructins, and Chapter 6 on crown acetals and other flexible non-glucose cyclooligosaccharides).




The data on the solution conformation of α-cycloaltrin is further substantiated by the unique crystal structure of this compound, which displays the alternating succession of the two different altrose conformations in the environment of water included in the crystal lattice. The molecular geometries of α-cycloaltrin in the solid-state and in solution closely resemble each other, the heavily hydrated X-ray-derived geometry obviously represents a frozen snapshot geometry of the solution state. In both cases, the molecular surface of this host displays no central cavity, but it is fully closed at the 6-$CH_2OH$ side of the macrocycles.

On the basis of these findings, α-cycloaltrin seemed not to be able to form inclusion complexes due to the lack of a central cavity. However, molecular dynamics simulations on α-cycloaltrin indicate the flexibility of this compound, with conformations being accessible which have central cavities and torus like shapes resembling the cyclodextrins. Indeed, the $K_{1:1}$ association constants of α-cycloaltrin and its larger ring homologs β- and γ-cycloaltrin with various *para*-substituted sodium benzoates have been determined by capillary electrophoresis. The data shows the formation of weak inclusion complexes by the cycloaltrins, with lowered stability and less pronounced shape selectivity for the inclusion of guest molecules than is observed for the cyclodextrins. Obviously, the data reflects the entropic penalty to be paid for the formation of cycloaltrin conformers featuring a central cavity to include the guest molecules. The next Chapter provides additional data and first unequivocal evidence on the *induced-fit*-type inclusion complexation by a flexible cyclooligosaccharide.

For details see Chapter 3, publications:

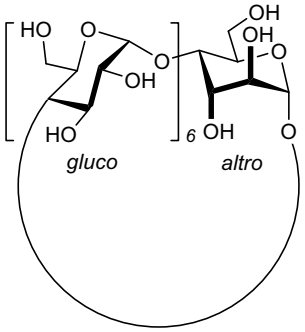
Synthesis, Structure, and Conformational Features of α-Cycloaltrin: a Cyclooligosaccharide with alternating $^4C_1$ and $^1C_4$ Pyranose Chairs.
Y. Nogami, K. Nasu, T. Koga, K. Ohta, K. Fujita, S. Immel, H. J. Lindner, G. E. Schmitt, and F. W. Lichtenthaler, *Angew. Chem.* **1997**, *109*, 1987-1991; *Angew. Chem. Int. Ed. Engl.* **1997**,*35*, 1899-1902.

Solution Geometries and Lipophilicity Patterns of α-Cycloaltrin.
S. Immel, K. Fujita, and F. W. Lichtenthaler,
*Chem. Eur. J.* **1999**, *5*, 3185-3192.

Inclusion Complexes of Cycloaltrins.
S. Immel, K. L. Larsen,
*unpublished results*.

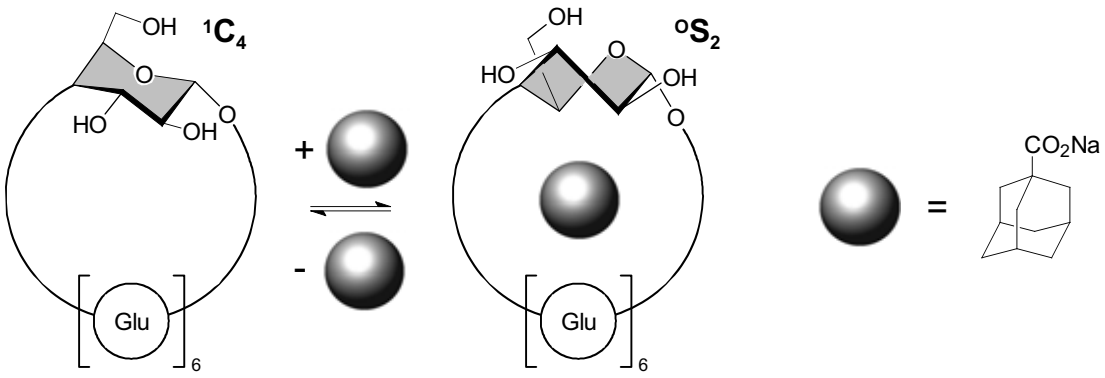
## 4. Mono-*altro*-β-cyclodextrin

Mono-*altro*-β-cyclodextrin, accessible from β-cyclodextrin via configurational conversion of one of the glucose units into altrose, is shown to be a flexible host undergoing a distinct conformational change within its altropyranose geometry upon intracavity inclusion of adamantane-1-carboxylate. The detailed analysis of the $^1$H NMR spectra in $D_2O$ at 35°C, and determination of all proton-proton coupling constants within the α-D-altrose pyranoid ring upon titration of the host molecule with the adamantane guest reveals distinct changes in the coupling parameters. Correlation of the measured data with calculated coupling constants derived from molecular dynamics simulations on the host revealed evidence of a conformational transition of the altrose ring within the macrocycle upon inclusion complexation. In the free host, the altrose units preferably adopt a $^1C_4$ chair geometry, whereas, in the complex, the conformational preferences are shifted towards the $^OS_2$ skew-boat form.




**mono-*altro*-β-cyclodextrin**

These results are further corroborated by a molecular dynamics and modelling study on the complex clearly revealing the cavity of the mono-*altro*-β-cyclodextrin being most regularly round shaped in the case of the $^{O}S_2$ altrose geometry in the macroring. Obviously, the conformational transition in this sugar unit is triggered by the incorporation of the ball-shaped adamantane guest upon entering the cavity to form the inclusion complex. However, the complex stability is significantly lower than the corresponding complex formed by β-cyclodextrin.




In this respect, mono-*altro*-β-cyclodextrin represents the first example for the flexible *induced-fit*-type molecular recognition of guest molecules by a cyclooligosaccharide, rather than the rigid *lock-and-key*-type inclusion complexation common to the cyclodextrins.

For details see Chapter 4, publication:

Guest-induced Conformational Change in a Flexible Host: Mono-*altro*-β-cyclodextrin.
K. Fujita, W.-H. Chen, D.-Q. Yuan, Y. Nogami, T. Koga, T. Fujioka, K. Mihashi, S. Immel, and F. W. Lichtenthaler, *Tetrahedron: Asymmetry* **1999**, *10*, 1689-1696.

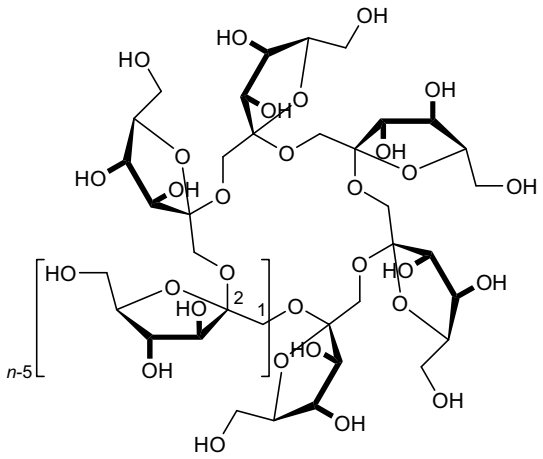
## 5. Cyclofructins and Cyclogalactofuranosides

The comparatively rigid pyranoid units of the amylose-derived cyclodextrins are contrasted by furanoid monosaccharides which contain a certain degree of an inherent flexibility, and cyclooligosaccharides composed thereof should portray this flexibility in their molecular recognition patterns. In this context, cyclofructins and some cyclogalactofuranosides (see below) have been subjected to a molecular modelling study to assess their structures, geometries, and potential for inclusion complexation.

Cyclofructins derived from inulin with six ($CF_6$) to ten ($CF_{10}$) β(1→2)-linked fructofuranose units were analyzed using molecular dynamics and *Monte-Carlo* simulations. It turned out, that the *spiro*-type anellation of fructofuranoses to a crown ether-type macrocyclic backbone renders these compounds highly flexible, although

some significant and unique conformational trends are retained for the entire series of ring size homologs: none of the cyclofructins retains full rotational symmetry, but in all cases the molecular symmetry is partially broken by an alternating inclination ("*up*" or "*down*") of furanoid units in relation to the macroring.

The discontinuous arrangement of the sugar units substantially lowers the strain energy of the cyclofructins, the energy-minimum geometries of $CF_6$, $CF_8$, and $CF_{10}$ exhibit $C_{n/2}$ rotational symmetry, while the odd-membered macrocycles, $CF_7$ and $CF_9$, adopt $C_1$ symmetry. Evidence for the relevance of the geometries obtained is derived from comparisons with the solid-state geometry of $CF_6$. Most notably, the cyclofructins represent another example of a cyclooligosaccharide with flexible units (or flexible linkages between the individual residues) displaying the conformational phenomenon of pseudorotation: structural rearrangements result in structures which appear to be rotated initial states only. Other examples along this vein are described in Chapter 3 (→ cycloaltrins) and Chapter 6 (→ crown acetals) of this work.



**cyclofructins**

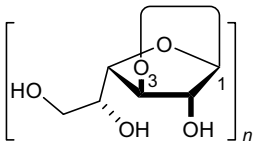
*cyclo*[D-Fru*f* β(1→2)]$_n$
(*n* = 6-10)

The molecular geometries and surfaces calculated for the energy-minimum structures establish a disk-type shape for $CF_6$, $CF_7$, and $CF_8$, whereas further ring enlargement to $CF_9$ and $CF_{10}$ leads to torus-shaped molecules with through-going cavities. Surface mapping of molecular lipophilicity patterns (MLPs) and the electrostatic potential profiles (MEPs) revealed crown ether-like properties of the cyclofructins, and a high potential for the complexation of metal cations. Both $CF_9$ and $CF_{10}$ display significantly hydrophobic central cavities accessible for the formation of inclusion complexes; studies towards the molecular recognition and inclusion of amino acids are described along with hydrophobic as well as electrostatic interactions in the complexes.

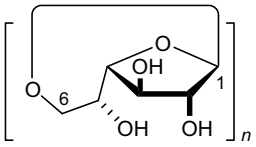
For details see Chapter 5, publication:

Cyclofructins with Six to Ten β(1→2)-linked Fructofuranose Units: Geometries, Electrostatic Profiles, Lipophilicity Patterns, and Potential for Inclusion Complex Formation.
S. Immel, G. E. Schmitt, and F. W. Lichtenthaler,
*Carbohydr. Res.* **1998**, *313*, 91-105.

As demonstrated for the cyclofructins, furanoid rings display a higher pseudorotational mobility than their pyranoid counterparts, and cyclooligosaccharides composed of furanoid sugar units are apt to be more flexible in their macrocycle. The same applies to the synthetically accessible cyclogalactins composed of β(1→3)- and β(1→6)-linked galactofuranose units which are likely to represent highly flexible host molecules for the *induced-fit*-type molecular recognition of potential guest molecules. Due to the rather modest yields in their preparation, the molecular geometries of the cyclogalactofuranosides composed of β(1→3)- and β(1→6)-linked galactofuranose units, i.e. *cyclo*[D-Gal*f* β(1→3)]$_n$ with $n$ = 4 and 5, and *cyclo*[D-Gal*f* β(1→6)]$_n$ with $n$ = 3 and 4, and their capabilities to form inclusion complexes were investigated by means of molecular dynamics and *Monte-Carlo* simulations. The latter compounds feature a significantly increased degree of flexibility, not the last due the larger number of atoms contained in the intersaccharidic linkages.




*cyclo*[D-Gal*f* β(1→3)]$_n$
($n$ = 4,5)




*cyclo*[D-Gal*f* β(1→6)]$_n$
($n$ = 3,4)

**cyclogalactofuranosides**

The flexibility of the macrocyclic backbone strongly favors bent and asymmetrical conformations over round geometries. Generation of the molecular surfaces of the global energy-minimum structures reveal disk-type shapes for these hosts without through-going central cavities, yet distinct indentations close to the O-2/O-3-groups, respectively. The molecular lipophilicity patterns prove these surface dents to be hydrophobic for the β(1→6)-linked cyclogalactins, whereas their β(1→3)-linked counterparts display an inverse situation with a hydrophobic outer core structure.

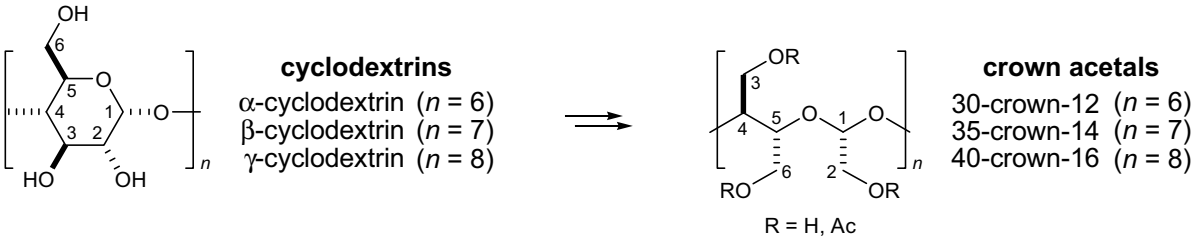
For details see Chapter 5, publication:

Conformations and Lipophilicity Profiles of some Cyclic β(1→3)- and β(1→6)-linked Oligogalactofuranosides.
H. Gohlke, S. Immel, and F. W. Lichtenthaler,
*Carbohydr. Res.* **1999**, *321*, 96-104.

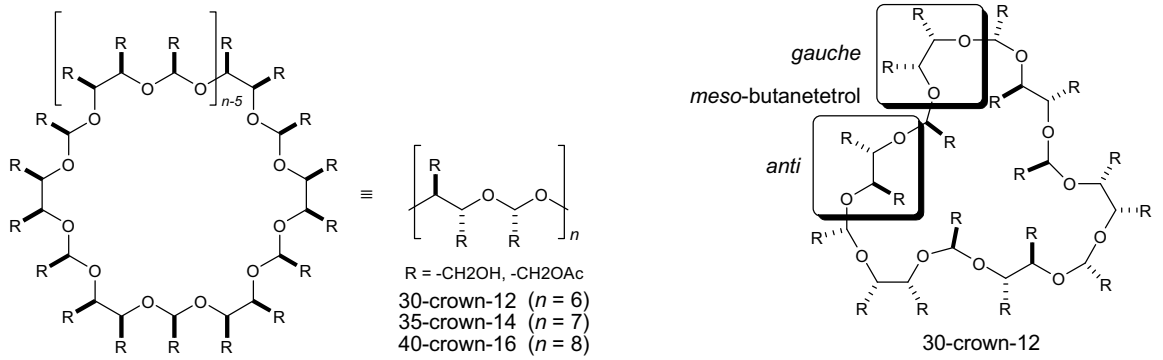
## 6. Cyclodextrin-derived Crown Acetals

An entirely different approach towards the generation of flexible macrocyclic hosts is demonstrated by the synthesis of cyclodextrin-derived crown acetals. Unlike crown ethers which have played a pivotal role in the development and understanding of supramolecular chemistry, cyclic compounds containing exclusively acetal oxygen atoms are rare and only a few examples have been reported so far; macrocycles of this type – as of now – are utter curiosities, and no attempts have been made previously to elucidate their structures and molecular geometries. A highly efficient

route towards the preparation of macrocyclic crown acetals derived from cyclodextrins is presented by the periodate oxidation and cleavage of the C-2 / C-3 bonds of the glucose residues: periodation, in situ reduction of the resulting polyaldehydes, and subsequent per-*O*-acetylation afforded macrocyclic poly-hydroxymethylene and poly-acetoxymethylene substituted macrorings as crystalline compounds in over-all yields of about 90%. Starting from α-, β-, and γ-cyclodextrins, the crown acetals of the 30-crown-12, 35-crown-14, and 40-crown-16 type were obtained. The macrorings are made up from six, seven, and eight alternating *meso*-butanetetrol and glycolaldehyde units, and thus represent achiral compounds.

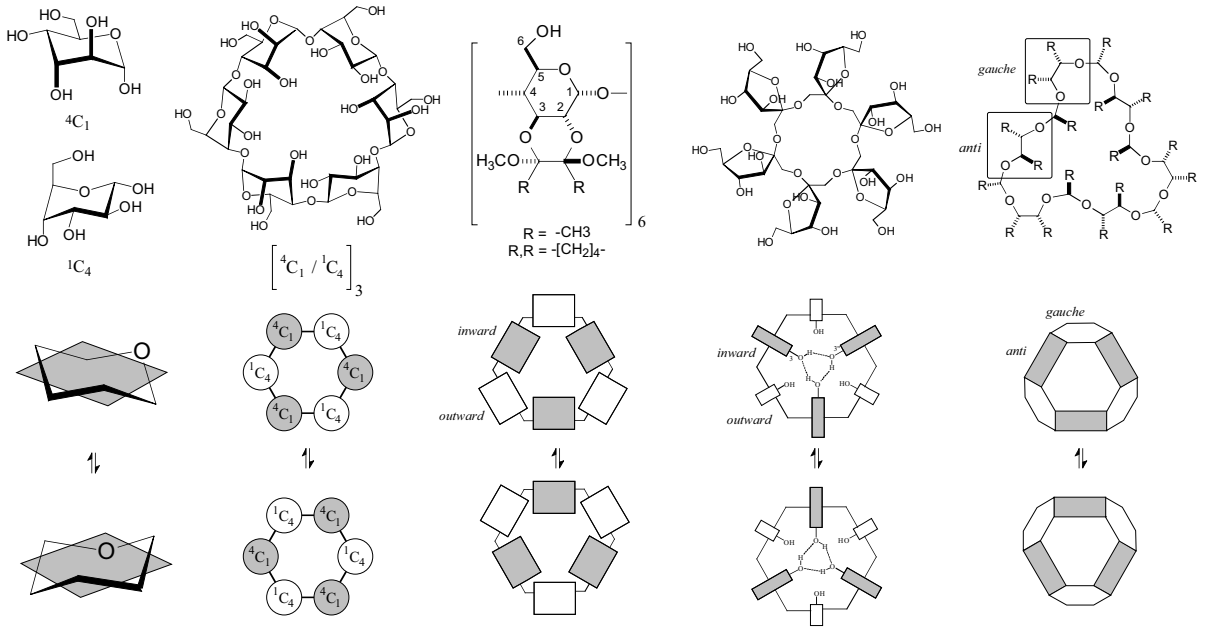



Although these large-ring crown acetals are anticipated to be highly flexible in solution, detailed temperature dependent NMR analysis revealed them to be less flexible than expected: at low temperatures, two signal sets are observed for the ring protons, revealing asymmetric conformations to be predominant in solution. These results are fully corroborated by the X-ray-derived solid-state structures of all poly-acetoxymethylene 30-crown-12, 35-crown-14, and 40-crown-16 acetals. In the crystalline state as well as in solution these macrorings preferentially adopt less symmetric conformations through an alternating succession of *gauche* and *anti* geometries of the constituent *meso*-butanetetrol residues. Conformational exchange occurs with an activation barrier of about $\Delta G^{\neq} \approx 60$ kJ/mol for the 30-crown-12 hexaacetal, the 40-crown-16 octaacetals appears to be even more flexible. However, the odd-membered β-cyclodextrin-derived 35-crown-14 heptaacetals represents the most flexible ring within this series of ring homologs.




With these results, not only is it the first time that the structures and molecular geometries of very large-ring crown acetals have been unraveled and reported in detail, but the crown acetals also provide another vivid example of the phenomenon

of pseudorotation in large-ring macrocycles and cyclooligosaccharides: breaking the symmetry of the rings reduces their strain energy, and conformational rearrangements of chemically equivalent, yet, geometrically distinctly different sub-units results in dynamic exchange processes. In all cases – i.e. for the cycloaltrins (Chapter 3), cyclofructins (Chapter 5), and the crown acetals (this Chapter) – the symmetry of the ring structures is halved, and even-membered rings appear to be less flexible than their next lower and higher odd-membered homologs.




α-D-altrose | α-cycloaltrin | "*extended cavity*" cyclodextrins | α-cyclofructin | 30-C-12 crown acetal (R = -$CH_2OH$, -$CH_2Oac$)

The last original publication provided in this Chapter lists the "*extended cavity*" cyclodextrins as an additional example of cyclooligosaccharides displaying the phenomenon of pseudorotation.

For details see Chapter 6, publications:

Synthesis and Molecular Geometry of an Achiral 30-Crown-12 Polyacetal from α-Cyclodextrin.
S. Immel, T. Nakagawa, H. J. Lindner, and F. W. Lichtenthaler,
*Chem. Eur. J.* **2000**, *6*, 3366-3371.

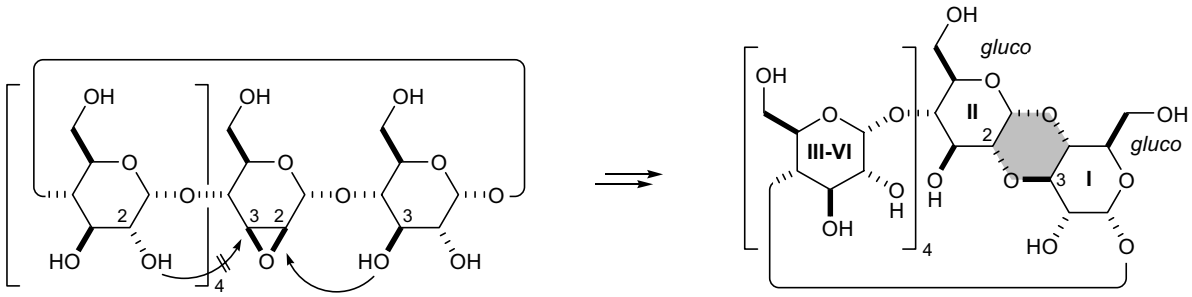
Hydroxymethyl-substituted Crown Acetals with 35-C-14 and 40-C-16 Skeletal Backbones: Synthesis and Molecular Geometries.
S. Immel, F. W. Lichtenthaler, H. J. Lindner, and T. Nakagawa,
*Tetrahedron: Asymmetry* **2001**, *12*, 2767-2774.

Large-ring Crown Acetals from Cyclodextrins.
T. Nakagawa, S. Immel, H. J. Lindner, and F. W. Lichtenthaler,
*Proc. 10th Int. Symp. Cyclodextrins* (Ed.: J. Szejtli), Mia Digital Publ., Ann Arbor, Michigan, **2000**, pp. 18-23.

Flexible Non-glucose Cyclooligosaccharides.
S. Immel,
*Proc. 10th Int. Symp. Cyclodextrins* (Ed.: J. Szejtli), Mia Digital Publ., Ann Arbor, Michigan, **2000**, pp. 24-31.

## 7. Rigidified Bridged Cyclodextrins

The introduction of flexibility into the cyclodextrins described so far, is opposed by the approach to increase the rigidity of the macrocycles through intramolecular bridging between the glucose residues. Although regioselective epoxide ring opening of $2^{I},3^{I}$-(2$^{I}$*S*)-anhydro-α-cyclodextrin through intramolecular attack of neighboring hydroxyl groups is expected to occur in the usual diaxial fashion to yield the *altro*-configured product, the diequatorial ring opening is observed exclusively. This approach yields $3^{I},2^{II}$-anhydro-α-cyclodextrin with a rigid glucopyranose-dioxane-glucopyranose tricyclic ring system incorporated into the backbone of the macrocycle.




**$2^{I},3^{I}$-anhydro-(2$^{I}$*S*)-α-cyclodextrin** **$3^{I},2^{II}$-anhydro-α-cyclodextrin**

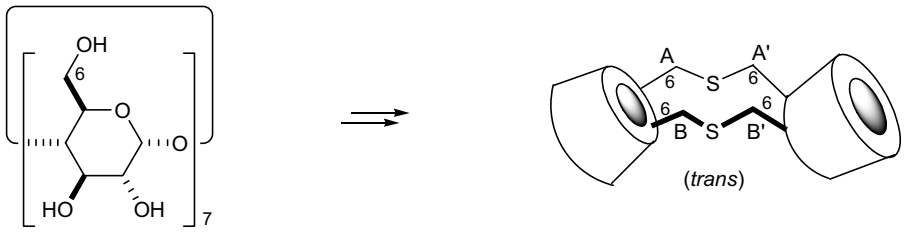
Molecular dynamics simulations in water were used to analyze the conformations of $2^{I},3^{I}$-(2$^{I}$*S*)-anhydro-α-cyclodextrin and both stereoisomers of the epoxide ring opening reaction. From these simulations, the observed *gluco*-configured product not only emerged as being thermodynamically more stable than the alternate *altro*-configured stereoisomer, but also being the favored product on geometrical considerations. Within the strait jacket of the macrocycle of $2^{I},3^{I}$-(2$^{I}$*S*)-anhydro-α-cyclodextrin, the intramolecular distances between the epoxide ring carbons and the hydroxyl groups of the adjacent glucose units, as well as their angle of attack obviously favors the unusual mode of ring opening.

The solid-state structure of the $3^{I},2^{II}$-anhydro-α-cyclodextrin · 3 *n*-PrOH nona-hydrate complex displays an inverted conicity of the macroring compared to the parent α-cyclodextrin, caused by contraction of the 2,3-OH side of the torus. In the environment of the crystal lattice, the three *n*-propanol molecules are distributed in the cavity of a dimeric unit of the host. Based on comparisons of the crystal structure with molecular dynamics simulations of this compound in water, the cyclodextrin derivative undergoes no significant conformational changes upon dissolution.

For details see Chapter 7, publication:

Two Stereoisomeric $3^{I},2^{II}$-Anhydro-α-cyclodextrins: A Molecular Dynamics and Crystallographic Study.
S. Immel, K. Fujita, M. Fukudome, and M. Bolte,
*Carbohydr. Res.* **2001**, *336*, 297-308.

In addition to the intramolecular bridging of cyclodextrins described above, intermolecular bridging represents an appropriate approach towards rigidified cyclodextrin dimers with extended cavities. Although cyclodextrin dimers and oligomers have been studied in the past, this work focuses on the option to introduce the shortest possible single-atom linkages between individual cyclodextrin units. In this context, β-cyclodextrin is transannularly disulfonylated at the $6^A$- and $6^B$-positions, and then converted to the corresponding $6^A$,$6^B$-diiodide and $6^A$,$6^B$-dithiol. Cross-coupling of the latter two species yields a single head-to-head coupled β-cyclodextrin dimer with two sulfur-linkers at adjacent 6-methylene carbons. Although this approach is likely to yield two stereoisomeric *cis*- and *trans*-coupled cyclodextrin dimers differing in the orientation of the macrorings relative to each other, only the latter *trans*-type ("*aversive*") linked product was observed and obtained in crystalline form.




**β-CD** **β-CD dimer**

NMR and X-ray analysis unequivocally revealed the type of linkage between both β-cyclodextrin rings in the new dimeric structure. For the first time, the solid-state structure of such a bridged cyclodextrin has been solved in the form of its hydrated methanol inclusion complex. The rather undistorted macrocycles feature almost parallel ring planes pointing away from each other, leaving the dimer with a "handcuff"-like appearance of approximate $C_2$ symmetry, the rotational axis of symmetry passing through the hexagon described by the $6^A$-C-S-$6^{A'}$-C and $6^B$-C-S-$6^{B'}$-C atoms. This "dimeric" host opens up the possibility to specifically form inclusion complexes with potential guest molecules of 1 : 2 stoichiometry. Moreover, the rather rigid linkage of this host molecule with two separated cavities may allow to study included guest molecules and their long-range interactions at well-defined distances.

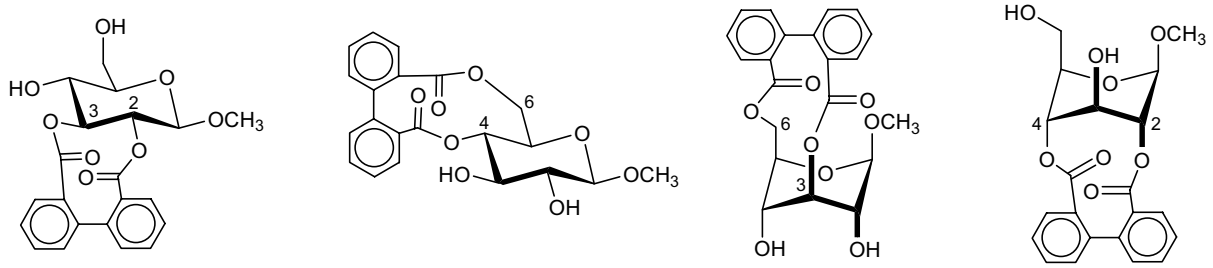
For details see Chapter 7, publication:

The First Successful Crystallographic Characterization of a Cyclodextrin Dimer: Efficient Synthesis and Molecular Geometry of a Doubly Sulfur-bridged β-Cyclodextrin.
D.-Q. Yuan, S. Immel, K. Koga, M. Yamaguchi, and K. Fujita,
*Chem. Eur. J.* **2003**, *9*, 3501-3506.

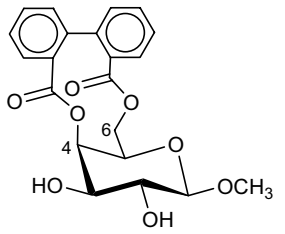
## 8. Molecular Modelling of Saccharides

This Chapter describes molecular modelling studies on various saccharides not related to the concept of creating more flexible or rigidified molecular host-guest systems. However, the studies presented are of interest for evaluating the chemical, stereochemical, conformational as well as biological properties of low-molecular weight saccharides.

Ellagitannins represent a class of polyphenolic natural products contained in higher plants, their common structural element being hexahydroxydiphenoyl (HHDP) substituted D-glucopyranosides with varying substitution patterns, and these tannin-components exhibit a broad range of biological activities. The axially chiral diphenoyl units occur with specific configurations depending on the positions of attachment. In order to evaluate the effects determining the mode of chirality, a number of model compounds such as methyl 2,3-, 4,6-, 3,6-, and 2,4-*O*-diphenoyl β-D-gluco-pyranosides were subjected to a molecular modelling study; for comparison the corresponding non-natural 4,6-*O*-diphenoyl β-D-galactoside was included in this work.



**methyl *O*-diphenoyl-β-D-glucosides**



**methyl 4,6-*O*-diphenoyl-β-D-galactoside**

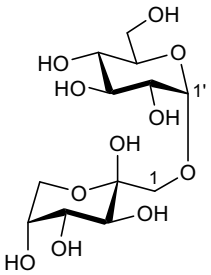
On the basis of molecular mechanics and dynamics simulations, the 10- to 12-membered rings containing the diphenoyl residues are far less flexible than expected: the rather rigid ester linkages transfer the chiral information of the saccharide units specifically into the diphenoyl units, the chiral scaffold of the saccharides excerpts a strong atropdiastereoselective effect onto the diphenoyl moieties. For the 2,3- and 4,6-*O*-diphenoyl bridged glucosides the (*S*)-diphenoyl configuration is energetically highly preferred, whereas for the 3,6- and 2,4-*O*-linked diphenoyl glucosides the opposite (*R*)-configuration is favored. All the results are in full accord with the absolute configurations found in the natural products, and thus provide explanations for the observed chirality on the basis of geometrical reasons. Notably, for the

unknown 4,6-*O*-diphenoyl galactoside this effect is reversed, and the (*R*)-form is proposed to be more stable.

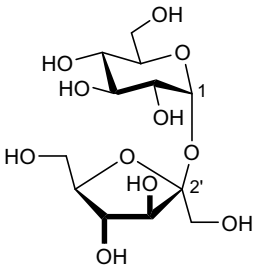
For details see Chapter 8, publication:

Atropdiastereoisomers of Ellagitannin Model Compounds: Configuration, Conformation, and Relative Stability of D-Glucose Diphenoyl Derivatives.
S. Immel, K. Khanbabaee,
*Tetrahedron: Asymmetry* **2000**, *11*, 2495-2507.

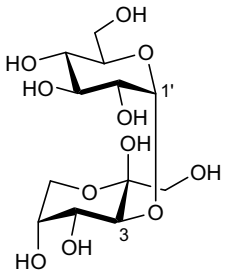
*Klebsiella pneumoniae* – the first bacterial species identified in this respect – and *Fusobacterium mortiferum* utilize not only sucrose (D-fructofuranosyl-β(2→1) α-D-glucopyranoside), but also its five linkage-isomeric α-D-glucosyl-D-fructoses trehalulose α(1→1), turanose α(1→3), maltulose α(1→4), leucrose α(1→5), and palatinose α(1→6) as energy sources. Growth on the sucrose isomers induced the expression of proteins identified as an $NAD^+$ and metal ion-dependent 6-phospho-α-glucosidases not present in sucrose-grown cells; the corresponding genes have been identified, cloned, expressed, and characterized. The enzymes catalyzed the hydrolysis of a wide variety of 6-phospho-α-glucosides including the above sucrose isomers, but not sucrose 6-phosphate itself.



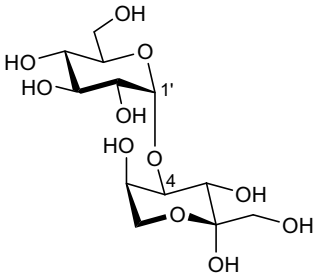
**β-*p*-Trehalulose**
α-D-Glc*p*-(1→1)-
β-D-Fru*p*



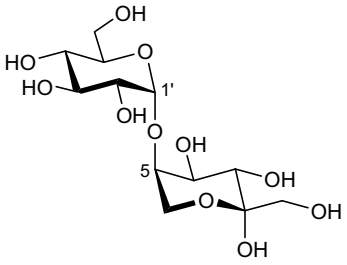
**Sucrose**
β-D-Fru*f*-(2↔1)-
α-D-Glu*p*



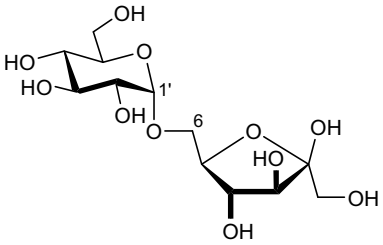
**β-*p*-Turanose**
α-D-Glc*p*-(1→3)-
β-D-Fru*p*



**β-*p*-Maltulose**
α-D-Glc*p*-(1→4)-
β-D-Fru*p*



**β-*p*-Leucrose**
α-D-Glc*p*-(1→5)-
β-D-Fru*p*



**β-*f*-Palatinose**
α-D-Glc*p*-(1→6)-
β-D-Fru*f*

Based on conformational analysis and molecular dynamics simulations of the six disaccharides and their glucosyl 6-phosphates in aqueous solution, significant differences in molecular shape and lipophilicity potential between sucrose and its isomers have been identified. The results explain the enzymatic differences in selectivity on the basis of the substrate properties.

For details see Chapter 8, publications:

Metabolism of Sucrose and Its Five Linkage-isomeric α-D-Glucosyl-D-fructoses by *Klebsiella pneumoniae*.
J. Thompson, S. A. Robrish, S. Immel, F. W. Lichtenthaler, B. G. Hall, and A. Pikis,
*J. Biol. Chem.* **2001**, *276*, 37415-37425.

Metabolism of Sucrose and Its Five α-D-Glucosyl-D-fructose Isomers by *Fusobacterium mortiferum*.
A. Pikis, S. Immel, S. A. Robrish, and J. Thompson,
*Microbiology* **2002**, *148*, 843-852.

## 9. Molecular Graphics

All molecular graphics contained within this work have been generated using the self-written *Molecular Architecture Modelling Program MolArch*$^+$. This program was developed for the purpose of simple automated analysis and 3D fitting of molecular structures, calculation of numerous geometry parameters, as well as providing the possibility to visualize organic and inorganic structures with different modes (wire models, capped-sticks, ball-and-stick models, CPK-type models). Solid-state crystal structures and packings (including thermal anisotropic ellipsoids and Hirshfeld surfaces), coordination polyhedra, molecular orbitals and surfaces with color-coded mapped properties, 3D grid and density data, and ribbon models of arbitrary (including non-protein) structures can be represented. Options are included to generate molecular animations for sets of molecular configurations along reaction coordinates or molecular dynamics simulations. *MolArch*$^+$ is fully interfaced to import and export a large variety of different file formats, and to produce high-quality color models with the *POVRAY* ("*Persistence of Vision*") ray-tracing program or any *VRML* ("*Virtual Reality Modelling Language*") viewing program. Chapter 9 gives an overview on the unique options provided by the *MolArch*$^+$ software.

For details see Chapter 9:

*MolArch*$^+$ *- Molecular Architecture Modelling Program.*
S. Immel,
*http://caramel.oc.chemie.tu-darmstadt.de/immel/.*