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Crystal structures of linear and cyclic oligosaccharides: An overview

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Abstract

The structural properties of cyclodextrins and of linear maltooligosaccharides of chain lengths 5 to 20 are discussed. The latter are prepared by enzymatic synthesis and characterized by circular dichroism spectroscopy. The crystal structure of a maltohexaoside and of cyclodextrin inclusion compounds are discussed in terms of polyiodide complexation and hydrogen bonding.

Although starch is one of the most abundant biological molecules, details of its three-dimensional structure are still not available. The reason is that starch, which consists of linear amylose and of the branched amylopectin, cannot be crystallized because it has no well-defined, globular molecular structure. It has only been possible to draw fibres of amylose, and X-ray diffraction patterns taken from these fibres have been interpreted in terms of a right handed double-helical structure where two parallel strands are intertwined (1). This model, however, is still under dispute and has recently been reinterpreted in the form of a left-handed double helix using new information based on electron diffraction and X-ray powder data (2).

If small molecules like iodine, alcohols or dimethylsulfoxide are added to an aqueous solution of amylose, inclusion compounds are formed where amylose is wound in a left-handed single stranded helix called V-amylose. It has a central cavity of about 5Å into which small molecules can be included. Structural models for these complexes are again based on X-ray fibre diffraction patterns, and consequently there are still uncertainties concerning details of the three-dimensional structure of V-amylose (ref. 3).

The main reason for these uncertainties is that in the fibres, amylose molecules are only in quasi-crystalline order, and the few obtainable X-ray reflections are insufficient to derive phase angles and to calculate electron density maps. Their interpretation relies heavily on model building which, per se, is biased by the experimentalist and can only subsitute for crystallographic methods if no crystals are available.

In one of our research projects, we intend to elucidate the structure of amylose at greater detail. We have worked out procedures to prepare oligosaccharides of defined chain lengths which can be used for crystallization experiments (refs. 4, 5). In a first X-ray structure analysis, we have determined the conformation of a malto-hexaoside in complex with polyiodide (ref. 6). It forms a left-handed, antiparallel double helix which is clearly different with respect to V-amylose or to the starch double helix. In addition, we have looked at cyclodextrin inclusion complexes, which can be considered as models for V-amylose. They crystallize readily in a form suitable for X-ray and even neutron diffraction experiments.

In the following, we describe our procedures to synthesize oligo-saccharides in quantities that are sufficient for crystallization experiments, their properties, the crystal structure of the maltohexaoside/polyiodide complex, and of cyclodextrin inclusion complexes.

THE PREPARATION OF MALTOOLIGOMERS OF DEFINED CHAIN LENGTHS

In initial attempts to synthesize large quantities of linear maltooligomers, we used the stepwise elongation of a starter molecule by potato phosphorylase or muscle phosphorylase B. As starters, we used p-nitrophenyl-α-D-maltopentaoside, which is commercially available and proved to have the optimum chain length for the phosphorylases. The p-nitrophenyl-moiety served as a convenient marker due to its absorption at 300nm. The reaction was optimized with respect to starter length, pH-value, and temperature. We usually obtained a charakteristic distribution of p-nitrophenyl-modified maltooligomers as shown in Fig. 1, but we noticed with some batches that the starter suffered from disproportionation, because the minimum chain length was not only the pentamer if we used malto-pentaoside as starter, but also the corresponding tetramer, trimer and dimer. The reason for this appears to be that the enzymes were not highly purified and contained an enzyme that disproportionates maltooligomers. The existence of this so-called D-enzyme was veryfied when we incubated the p-nitrophenyl-maltopentaoside with the raw extract of potato phosphorylase.

The synthesis of maltooligomers with phosphorylase finally proved not to be suitable for the production of large quantities because we had severe problems with the separation of the individual molecules (Fig. 1) by preparative HPLC. Consequently, we changed the method and used cyclodextrin-glucosyltransferase in the production of maltooligomers of defined chain length (8). The advantage is that a suitable

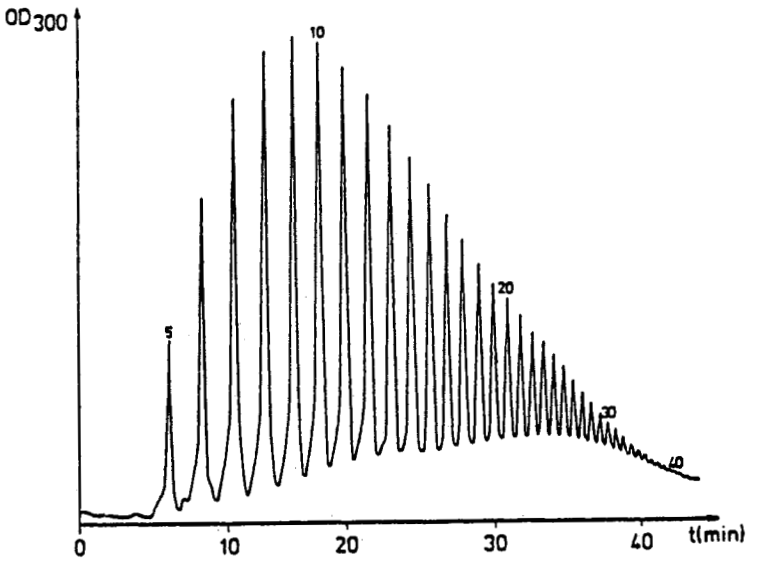


Fig. 1 HPLC-profile of 4-nitrophenyl-α-D-maltooligomers synthesized with potato phosphorylase. Stationary phase: Hypersil APS-2,5µm, acetonitrile/water linear gradient 75:72. The numbers at the peaks indicate the degree of polymerization (DP) of the oligomers. Taken from ref. 7.

starter molecule is elongated by 6,7, or 8 glucoses, depending on the cyclodextrin (α, β or γ) that is used in this reaction. P-nitrophenyl-α-D- maltopentaoside and unsubstituted maltopentaose were again suitable starters, and the reaction with α-cyclodextrin produced mainly the respective undecamer and heptadecamer, and small amounts of other chain lengths. The reaction is followed by HPLC. If the optimum concentration of the desired products is obtained, unused cyclodextrin is precipitated with tetrachloroethane. With p-nitrophenyl-substituted oligomers, the supernatant is applied to a column-containing β-cyclodextrin polymer, which forms inclusion complexes with the p-nitrophenyl moiety and serves to separate the different chain lengths. A final preparative HPLC of the different fractions from this column produces pure p-nitrophenyl-substituted (and unsubstituted) maltooligomers with defined chain lengths.

CHARACTERIZATION OF THE INCLUSION PROPERTIES OF THE UNSUBSTITUTED MALTOOLIGOMERS OF DIFFERENT CHAIN LENGTHS

In order to study the inclusion formation of the obtained maltooligomers, we used guest molecules with absorption in the UV or visible part of the spectrum and monitored the inclusion by the maltooligomers with circular dichroism. In Fig. 2, some of these spectra are shown which indicate that the affinity to the guest molecules

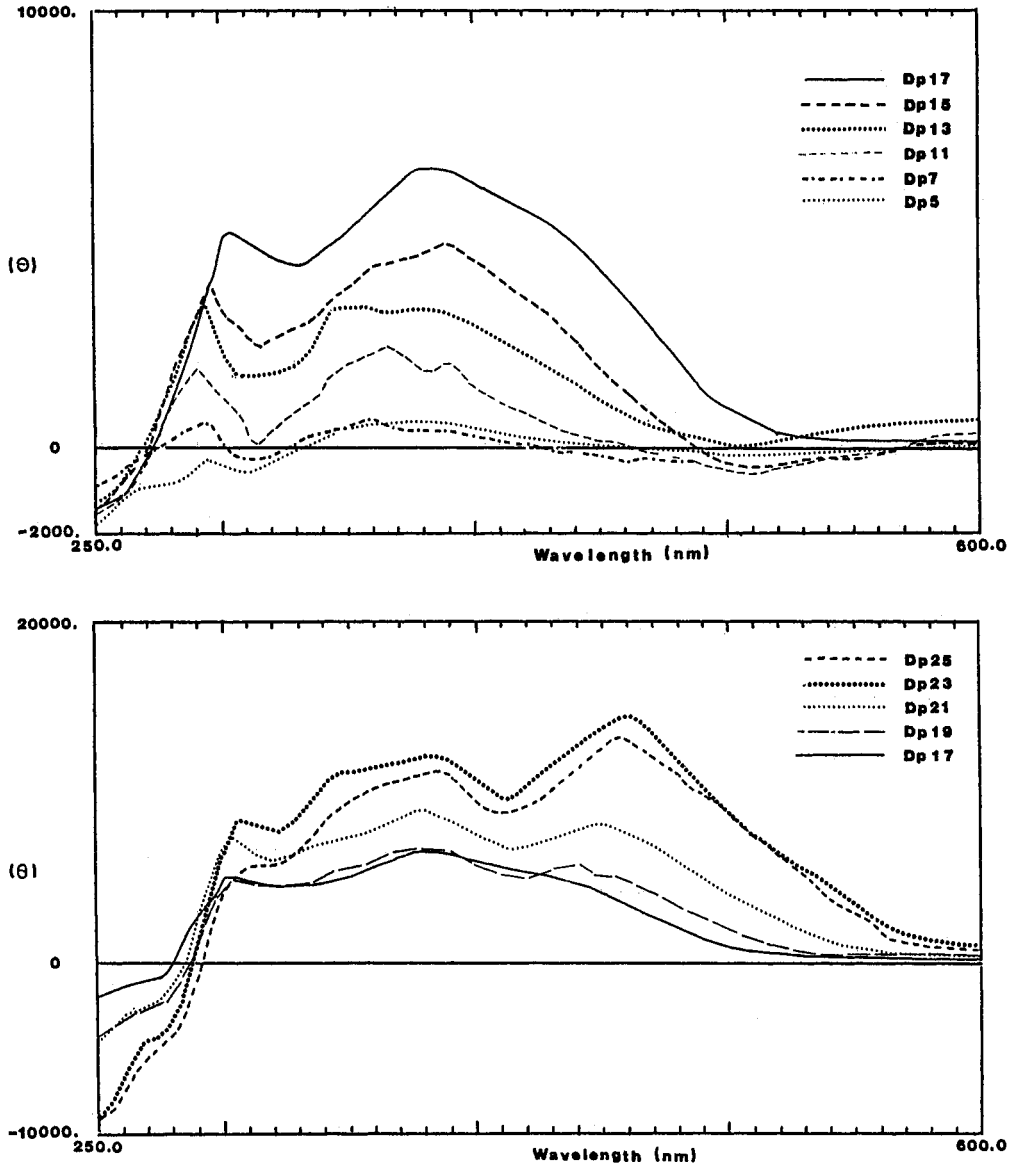


Fig. 2 Circular dichroism spectrum of I_2/KI complexed by maltooligosaccharides with increasing chain lengths (R. Herbst, (to be published).

increases with increasing chain length. We assume that the maltooligomers probably form helical structures in aqueous solutions which can accommodate the respective guest molecules in their cavity. This produces a local asymmetry which is detected by the circular dichroism. Comparison with cyclodextrin shows that the inclusion by linear maltooligomers has a lower affinity, in agreement with the more flexible structure of the linear maltooligomers relative to the rigid structure of the cyclodextrins.

CRYSTAL STRUCTURE OF THE COMPLEX (p-nitrophenyl-α-D-maltohexaoside)$_2$-$Ba(I_3)_2 .27H_2O$

Numerous attempts were made to crystallize the series of commercially available p-nitrophenyl-α-D-maltooligomers with chain lengths 3-7. We did not use the unsubstituted oligomers to avoid mutarotation of the reducing end group. Based on our experience with the cyclodextrins, we did not only try to crystallize the pure p-nitrophenyl-α-D-maltooligomers from aqeous solution, but we also added smaller molecules like iodine/iodide, alcohols, fatty acids etc., and we tried a number of different salts of the iodides. One of these attempts was successful and we obtained brown, single crystals of composition (p-nitrophenyl-α-D-maltohexaoside)$_2 \cdot Ba(I_3)_2 \cdot 27H_2O$ (ref. 6).

The space group of these crystals is orthorhombic, $P2_12_12_1$ and unit cell constants are a=33.73, b=29.21, c=14.44Å. More than 10.000 X-ray diffraction data were collected and the structure determined by a combination of Patterson and Difference-Fourier methods and refined to R=0.092 for the 7590 reflections above 3σ. The triiodide molecules are linear and arranged along the crystallographic c-axis as an infinite zig-zag chain with interunit angles from 121° to 166°, see Fig. 3.

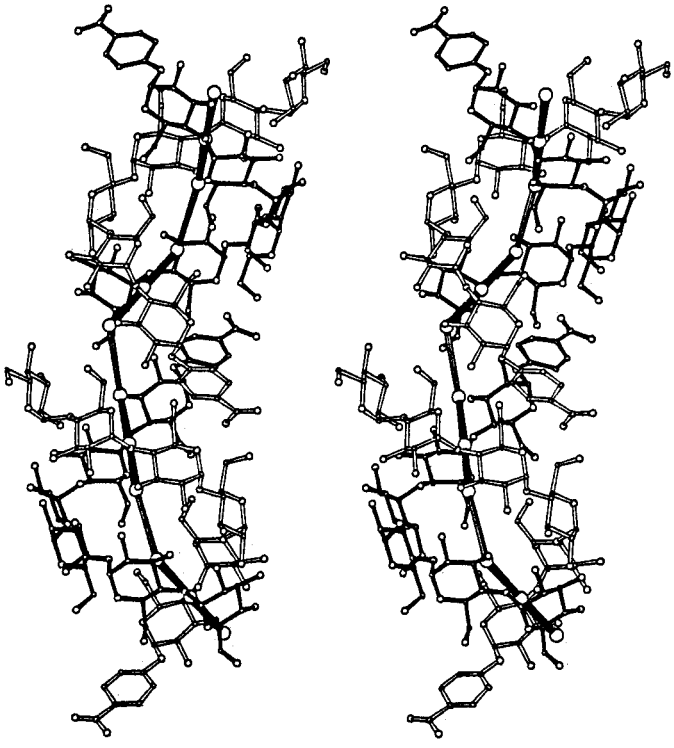


Fig. 3 Stereo view of the complex (p-nitrophenyl-α-D-maltohexaoside)$_2 \cdot Ba(I_3)_2$ $27H_2O$ (Ref. 6). Shown are two asymmetric units along the crystallographic c-axis, water molecules and Ba^{2+} are not drawn.

The structures of the two molecules of p-nitrophenyl-α-D-maltohexaoside in the asymmetric unit resemble cleaved cyclodextrins distorted in the form of lock-washers with left-handed screw-sense; all the 12 glucoses are in the $^4C_1$-chair form. Two lock-washers in opposite directions are wrapped around two I_3^-ions to form a left-handed antiparallel double helix. This double helix is stabilized by van der Waals interactions with the polyiodide chain, as observed with polyiodide complexes of amylose and of cyclodextrins, and by intramolecular, interresidue as well as intermolecular O(2)· · · · ·O(3') hydrogen bonds. The glucoses in the centres of the two maltohexaoside molecules are more regularly arranged than those at the ends. They were used to construct mathematically an amylose antiparallel double helix with 2 times 8 glucoses per turn, and a pitch height of 18.64Å.

In the crystal structure, the adjacent double helical complexes related by the 2_1-screw symmetry along c are arranged such that an "infinite" double helix is formed. It is stabilized by stacking interactions between the p-nitrophenyl groups, by hydrogen bonded water molecules serving as intermolecular bridges, by interactions between I_3^- units, and by coordination of Ba^{2+} to four different maltohexaoside molecules.

All except one of the 27 water molecules in the asymmetric unit are in direct hydrogen bonding contact with the double helix. There is a characteristic systematic hydration scheme such that glucose atoms O(2), O(3) and/or O(5), O(6) chelate water molecules to form 5-membered cyclic structures. This motif in glucose hydration is so systematic that it will probably occur in other heavily hydrated crystalline amylose fragments and, above all, in aqueous solution.

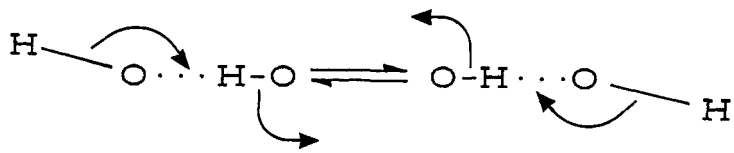
α-Cyclodextrin Polyiodide Complexes are Good Models for Starch-Iodine
In the complexes formed between α-CD and the Li^+ and Cd^{2+} salts of I_3^-, the α-CD molecules are stacked like coins in a roll, and the channel-like cavity is filled with polyiodide; the cations (and water molecules) are located between the stacks. The polyiodide is disordered $(I_2 \cdot I_3^-)_n$ in the Li^+-complex and $(I_5^-)_n$ in the Cd^{2+} complex. The I_2, I_3^- and I_5^- units are closer together than van der Waals contacts, suggesting that there is considerable charge transfer between them, in agreement with the deep blue to black color of these complexes. Since the width of the central cavities in V-amylose and in α-CD are comparable, we assume that the polyiodide chain in blue starch-iodine is similar to that observed in the α-CD complexes (9).

Cyclodextrins as models for understanding hydration phenomena.
If cyclodextrins are crystallized from pure water, they form different hydrates depending on conditions. The most preferred hydrates are

α-CD·6H_2O, β-CD·11H_2O and γ-CD·16H_2O. They feature numerous O-H···O hydrogen bonds, which form extended networks. These were studied by neutron diffraction, so that the positions of the H-atoms could be determined with high precision and permitted to define all the hydrogen bonds.

The hydrogen bonds in the α- and γ-CD hydrates are very well determined (10, 11). The α-CD ring of 6 glucoses is distorted such that one of the glucoses is rotated out of the ring formed by the others. This rotation is so severe that the intramolecular, interglucose hydrogen bonds O(2)···O(3') are broken for this glucose, but they are maintained for the other glucoses. In γ-CD, these hydrogen bonds are formed between all of the 8 glucoses. They obviously stabilize the circular structure of the cyclodextrins, which, as shown by circular dichroism measurements, is less rigid for the α-CD than for the other two cyclodextrins.

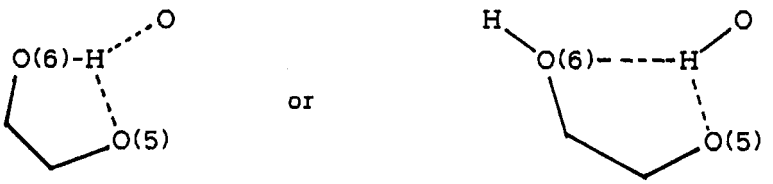
In β-CD-11 H_2O, the intramolecular, interglucose hydrogen bonds are all disordered, and of type O(2)-(1/2H)···(1/2H)-O(3'), Fig. 4. They where called flip-flop hydrogen bonds, because they can be described as the sum of two states which are in equilibrium (12),



Since the O(2), O(3) hydroxyl groups are in hydrogen bonding contact with water molecules which are also partly disordered, the flip-flop hydrogen bonds form extended chains. If the β-DC·11 H_2O crystals are cooled, a phase transition occurs at 227K. Neutron diffraction data collected below this temperature showed that the flip-flops are now ordered such that extended, cooperative hydrogen bonds are formed in which all the O-H· · ·O interactions point in the same direction (homodromic), O-H· · ·O-H· · ·O-H· · ·O-H (13). Quasielastic neutron scattering studies on crystalline β-CD·11 H_2O have provided further evidence for the dynamic nature of the flip-flop hydrogen bonds, and indicated that the jump rate between the two states is of the order $2 \cdot 10^{10}$ to $2 \cdot 10^{11}$ sec^{-1} (14).

As in (p-nitrophenyl-α-D-maltohexaoside)$_2$·Ba$(I_3)_2$·27H_2O, numerous CD-glucose residues chelate with water molecules or hydroxyl groups of neighbouring CD molecules hydrogen bonded to O(5) and O(6) of the same glucose. It was shown by neutron diffraction data that

these chelated arrangements represent three-center hydrogen bonds of the type



but never "chains" O(6)-H···O-H···O(5) (ref. 15).

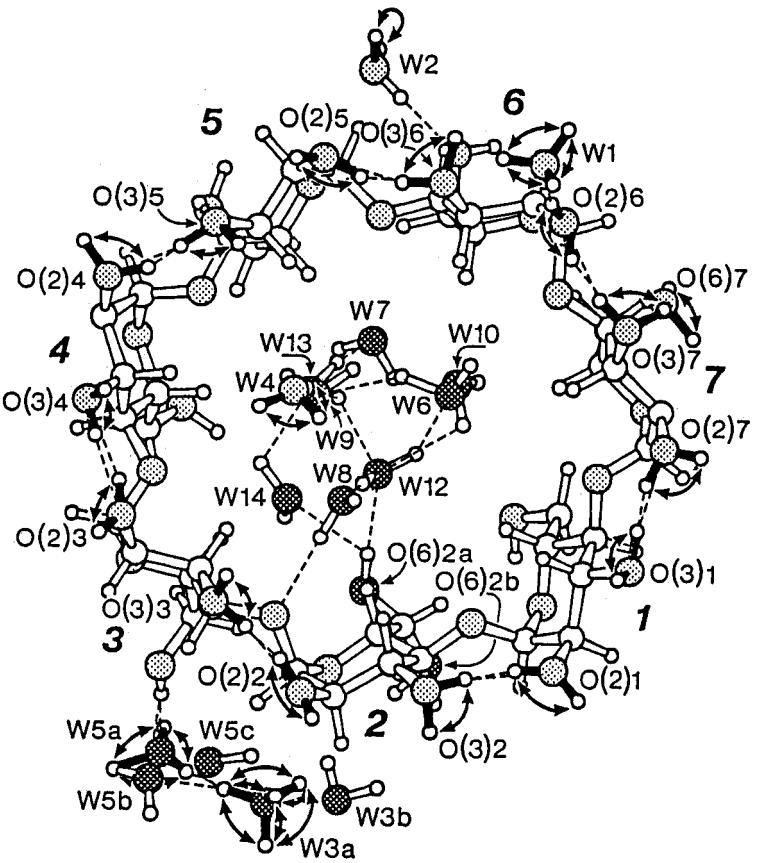


Fig. 4 Section of the crystal structure of β-cyclodextrin·11H_2O at room temperature as determined by neutron diffraction (14). Light shading: fully occupied oxygen positions; dark shading: partially occupied oxygen positions; solid bonds: orientationally disordered O-H groups involved in flip-flop hydrogen bonds; curved double arrows indicate jumps between the two different flip-flop states.

Acknowledgements

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REFERENCES

1. H.-C. Wu and A. Sarko, Carbohydr. Res. 61, 7-25 (1978) and ibid. 61, 27-40 (1978).

2. A. Imberty, H. Chanzy, S. Perez, A. Buleon and V. Tran, J. Mol. Biol. 201, 365-378 (1988).

3. T. L. Bluhm and P. Zugenmaier, Carbohydr. Res. 89, 1-10 (1981).

4. C. Niemann, R. Nuck, B. Pfannemüller and W. Saenger, Carbohydr. Res. 197, 187-196 (1990).

5. C. Niemann, W. Saenger, B. Pfannemüller, W. D. Eigner and A. Huber, in R. B. Friedman, Ed. Biotechnology of Amylodextrin Oligosaccharides, ACS Sympos. Series No. 458, pp. 189-204 (1991).

6. W. Hinrichs and W. Saenger, J. Amer. Chem. Soc. 112, 2789-2796 (1990).

7. C. Niemann, W. Saenger, R. Nuck and B. Pfannemüller, Carbohydr. Res. 215, 15-23 (1991).

8. C. Niemann, W. Saenger and B. Pfannemüller, Carbohydr. Res. 226, 119-130 (1992).

9. M. Noltemeyer and W. Saenger, Nature 259, 629-632 (1976); J. Amer. Chem. Soc. 102, 2710-2722 (1980).

10. B. Klar, B. E. Hingerty and W. Saenger, Acta Cryst. B36, 1154-1165 (1980).

11. J. Ding, Th. Steiner, V. Zabel, B. E. Hingerty, S. A. Mason and W. Saenger, J. Amer. Chem. Soc. 113, 8081-8089.

12. W. Saenger, Ch. Betzel, B. E. Hingerty and G. M. Brown, Nature 296, 581-583 (1982).

13. V. Zabel, W. Saenger and S. A. Mason, J. Amer. Chem. Soc. 108, 3664-3673 (1986).

14. Th. Steiner, W. Saenger and R. E. Lechner, Mol. Phys. 72, 1211-1232 (1991).

15. Th. Steiner, S. A. Mason and W. Saenger, J. Amer. Chem. Soc. 113, 5676-5687 (1991).