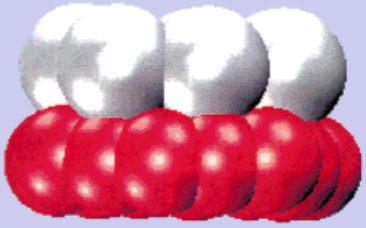
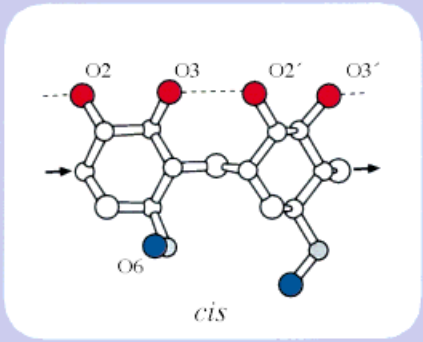
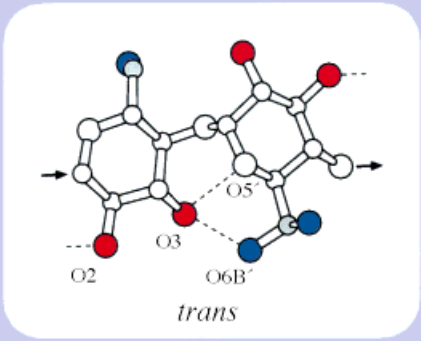
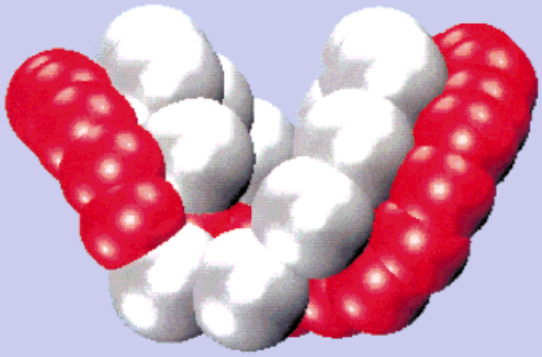
Band Flip as New Structural Motif in Amylose



In the well–known cyclodextrins, all glucose units are *cis*-oriented and connected by O3···O2′ hydrogen bonds forming cyclically closed bands. In larger cyclodextrins (cycloamyloses) with 10 and more glucoses, strain induced in the macrocycle is relieved by two 180° flips to *trans* orientation between diametrically opposed glucose units. These two "band flips" are stabilized by hydrogen bonds (O3···O5′ and O3···O6′), and lead to butterflylike structures. In the figures, cyclodextrins with 7 and 14 glucoses are shown; atoms O2, O3 are red, O6 blue, C6 grey, arrows mark chain direction, and dashed lines indicate hydrogen bonds.







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Strain-Induced "Band Flips" in Cyclodecaamylose and Higher Homologues**

Joël Jacob, Katrin Geßler,* Daniel Hoffmann, Haruyo Sanbe, Kyoko Koizumi, Steven M. Smith, Takeshi Takaha, and Wolfram Saenger*

Cycloamyloses (CAs) are cyclic molecules composed of $\alpha(1-4)$-linked glucoses in $^4C_1$ chair conformation. The smallest members, α-, β-, and γ-cyclodextrin (cyclohexa-, cyclohepta-, and cyclooctaamylose; CA6, CA7, and CA8, respectively), are cone-shaped and form inclusion complexes.[1] Because all glucose units are *cis*-oriented, O2 and O3 hydroxyl groups are on the wide side of the cone, and the O6 hydroxyl groups are on the narrow side. This gives rise to a bandlike cyclic structure. The central, hydrophobic cavity is coated with C3–H and C5–H atoms, and glycosidic O4 oxygen atoms. The cone structures are stabilized by intramolecular hydrogen bonds ($O2(n)\cdots O3(n-1)$) between the adjacent *cis* glucose residues. The exocyclic torsion angles (χ^5 O5-C5-C6-O6) are preferentially $-$*gauche*, but if O6 is hydrogen bonded to a guest molecule, this torsion angle may adopt the $+$*gauche* form.

From CA6 to CA8 the size of the annulus increases, with a concomittant decrease of curvature, and the coplanar O4 atoms form regular hexagons, heptagons, or octagons. For CA9 (δ-cyclodextrin), however, the O4 atoms describe an ellipse distorted into the shape of a boat.[2] The hydrogen-bonded O2 and O3 hydroxyl groups are at the wider "hull" side and arranged in a circle as observed for CA6 to CA8, but with small indentations. As indicated by molecular modeling, the distorted boat-shaped structure of CA9 is enforced by steric strain resulting from the size of the larger macrocycle.[3]

Is there a limit where steric strain induces conformational instability if the cyclodextrin macrocycle is further enlarged? Larger CAs became available by preparative scale treatment of amylose with cyclodextringlucanotransferases.[4] ^{13}C NMR spectroscopy on the series from CA6 to CA26[5] showed only one sharp signal for each of the six glucose carbon atoms indicating that glucose residues of the individual molecules are identical on the NMR time scale. Increasing the macrocycle size mainly affects signals of the C1 and C4 atoms, while resonances of the other carbons are only marginally influenced. For CA6 to CA8, C1 and C4 signals occur at $\delta \approx 102.4$ and $\delta \approx 81.8$, respectively; for CA10 and the higher homologues, they shift to $\delta \approx 100.2$ and $\delta \approx 78.3$, suggesting some as yet undefined structural differences. The signals for CA9 are intermediate, C1 at $\delta = 100.9$ and C4 at $\delta = 79.2$. The change in C1 and C4 signals between CA8 to CA10 indicates a clear distinction between two structural types. According to its ^{13}C NMR signals, CA9 could occur in both forms, the observed singlets being indicative of rapid structural changes well below the millisecond time range.

CA10 and CA14 (ε- and ι-cyclodextrin or cyclodeca- and cyclotetradecaamylose) crystallized from aqueous solution as 20.3 and 27.3 hydrates, respectively. X-ray analyses[6] show that both crystals belong to the monoclinic space group *C*2 with half a molecule in the asymmetric unit; the second half is related by crystallographic symmetry. The molecular shapes of CA10 and CA14 are very different than those of the smaller CA6 to CA9 (Figures 1 and 2). This is due to the flipping of

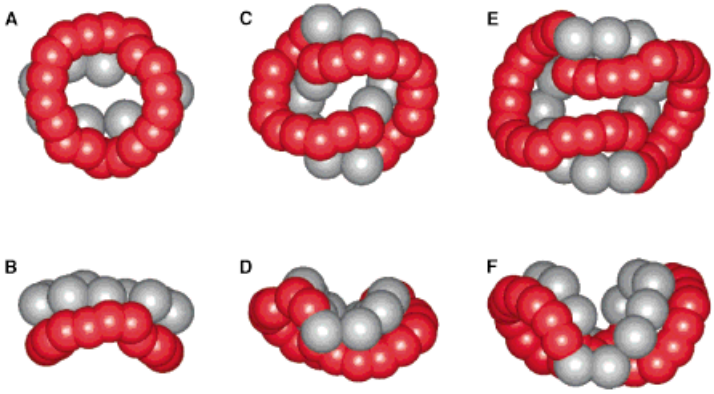

Figure 1. Schematic representation each in top and side views of the molecular structures of CA9 (A and B, respectively), CA10 (C and D, respectively), and CA14 (E and F, respectively). In Figures 1C and 1E the band flips occur where the course of the O2, O3, and C6 atoms is broken. For clarity, only O2, O3 (red), and C6 (grey) atoms are drawn. For numbering of O2, O3 hydroxyl groups, see Figure 4.

about 180° in *trans* orientation of two diametrically opposed glucoses, so that the ring of intramolecular $O2(n)\cdots O3(n-1)$ hydrogen bonds (for atom numbering see ref. [7,8]), which is still present in CA9, is disrupted (Figures 1C,E and 3A,B). As a consequence, the molecules are clearly divided into two halves connected at the flip sites called "band flips", as the typical band structure of amylose is interrupted; the central cavities are no longer open and round as in the smaller homologues but resemble narrow grooves. In a pictorial description, the molecules adopt the shape of a butterfly in which the wings are formed by cyclodextrin-like structures and the conformational band flips are located at the body.

There are significant conformational differences between CA10 and CA14. In CA10 the flipped glucose unit 1 is stabilized in orientation by a three-center hydrogen bond with O3(5′) as donor, namely $O3(5')\cdots O5(1)$ 3.21 Å and $O3(5')\cdots O6(1)B$ 2.74 Å; O6(1)B adopts the less preferred $+$*gauche*

[*] Dr. K. Geßler, Prof. Dr. W. Saenger, Dipl.-Chem. J. Jacob
Institut für Kristallographie der Freien Universität
Takustrasse 6, D-14195 Berlin (Germany)
Fax: (+49) 30-838-6702
E-mail: gessler@chemie.fu-berlin.de
Dr. D. Hoffmann
GMD-SCAI, Schloss Birlinghoven, Sankt Augustin (Germany)
H. Sanbe, Prof. K. Koizumi
School of Pharmaceutical Sciences, Mukogawa Women's University, Hyogo (Japan)
Prof. S. M. Smith
Institute of Cell and Molecular Biology, University of Edinburgh (UK)
Dr. T. Takaha
Biochemical Research Laboratory, Ezaki Glico Co., Ltd., Osaka (Japan)

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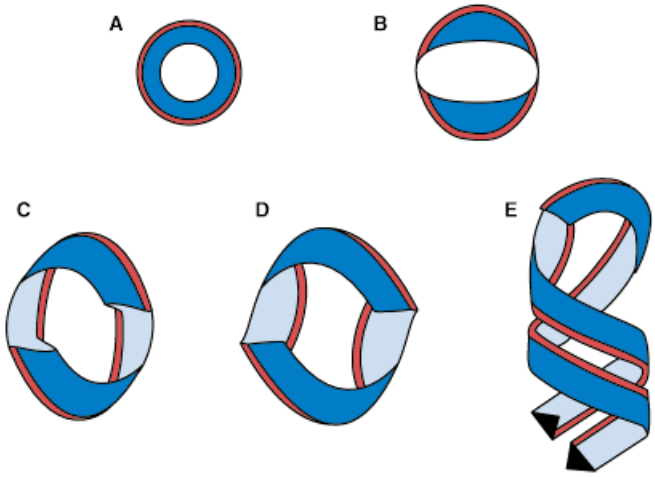

Figure 2. Illustration of folding schemes, thick red and thin black lines connect O2 to O3 and O6 atoms, respectively. Shown are annular, unstrained CA7 (A) and strained CA9 (B), and elliptically distorted CA10 (C), CA14 (D) and the proposed antiparallel left-handed, double helical form (only half a molecule) for larger cycloamyloses (E). The band flips in CA10, CA14 and larger CA occur where the red lines are broken.

form (Figure 4A). Between glucose residues 1 and 2, a kink increases the O2(2)···O3(1) distance to 3.94 Å, so this hydrogen bond is broken, but an unusual hydrogen bond between the O6 hydroxyl groups of these glucose units (O6(1)A···O6(2)B 3.08 Å) is formed and contributes to the stabilization of the kink (Figure 4A). The remaining glucose residues (2 to 5) are in cyclodextrin-like arrangement with interglucose O2(n)···O3($n-1$) hydrogen bonding distances in the range 2.89 to 3.03 Å.

In CA14, the flipped glucose 1 is held in orientation by the three-center hydrogen bonds O3(7′)···O6(1)B (3.05 Å) and O3(7′)···O5(1) (3.32 Å) reminiscent of the geometry around the flip site in CA10 (Figure 4B), with O6(1)B in the less preferred +*gauche* orientation. There is also a kink which, in contrast to CA10, does not directly follow the band-flip site but occurs between glucose residues 5′ and 6′, and is stabilized by a long (weak) O2(6′)···O3(5′) hydrogen bond of 3.40 Å. All other O2(n)···O3($n-1$) hydrogen bonds are in the common range (2.75–2.89 Å).

The band flip is best described by torsion angles ϕ and ψ commonly used to define oligosaccharide conformation.[9] They are in the normal range for the O2(n)···O3($n-1$) hydrogen-bonded glucose residues in the two halves ($\phi = 94$–110°, $\psi = 97^\circ$–135°). However, for the flipped glucose residues these torsion angles are $\phi = 84^\circ$ and $\psi = -65^\circ$ between residues 5′ and 1 in CA10 and $\phi = 82^\circ$, $\psi = -69^\circ$ between residues 7′ and 1 in CA14. In spite of these structural peculiarities, all glucose residues in CA10 and CA14 are in $^4C_1$ conformation and unstrained as indicated by Cremer and Pople[10] puckering parameters (not shown), and by the angles O4(n)···O4($n-1$)···O4($n-2$) subtended by O4 atoms (127–146° for CA10 and 132–145° for CA14).

The crystal packing of the butterflylike molecules CA10 and CA14 is very similar and of the channel type frequently observed for the smaller ring-shaped cyclodextrins and the related, chemically modified (cyclo(1–4)-α-D-rhamnopyranosyl-(1–4)-α-L-rhamnopyranoso)decasaccharide.[11] The CA10 and CA14 molecules are stacked head-to-tail along the crystallographic twofold rotation axes (parallel to the *b* axis), which have comparable lengths (9.981 Å for CA10 and 10.138 Å for CA14), implying that the differences in molecular size are accounted for by differences in the dimensions of the *a* and *c* axes. In contrast to the common channel structures, however, the cavities of CA10 and CA14 are so narrow that no continuous channel filled by guest molecules can form. The intermolecular contacts between the stacks are reminiscent of those observed in cage-type cyclodextrin packing motifs, and the butterflylike shape of the CA10 and CA14 molecules

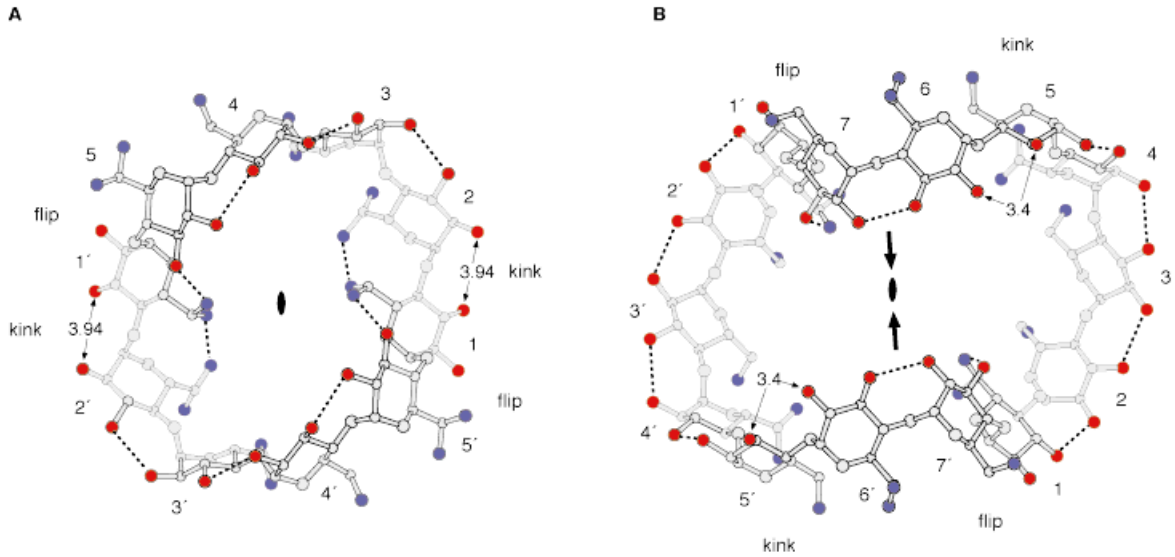

Figure 3. Molecular structures of CA10 (A) and CA14 (B). For clarity water molecules are omitted. Crystallographic twofold rotation axes are marked (⧫), glucose residues in the two asymmetric units are denoted with primed and unprimed numbers. O2 and O3 are red, O6 blue. O6 oxygen atoms of glucoses 1–3 and 5 in CA10 and 1, 4, 6 in CA14 are doubly disordered. Dotted lines suggest possible hydrogen bonds with smaller O···O distances than 3.5 Å. In CA14 the arrows in bold in the center indicate how glucose residues might approach each other in larger cycloamyloses to form antiparallel double helical structures with hydrogen bonds between O2 and O3 hydroxyl groups (see also Figure 2E). Drawn with the program MOLSCRIPT.[19]

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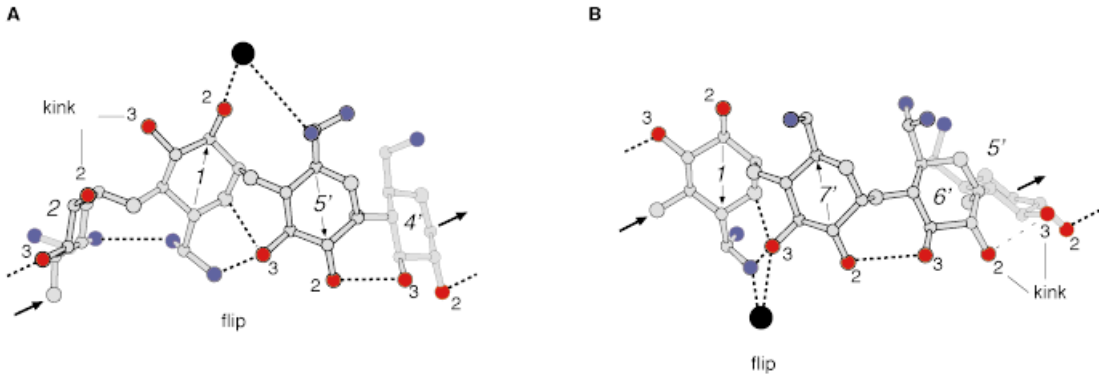

Figure 4. Structural details of kink and bond-flip sites in CA10 (A) and CA14 (B) viewed from the center of the molecules. Water oxygen atoms are represented in pink; O2, O3 are numbered and red, O6 are blue. The kink in CA10 (A) is stabilized by an unusual hydrogen bond between O6 hydroxyl groups (O6(1)A $\cdots$ O6(2)B 3.06 Å). The O2(2) $\cdots$ O3(1) distance at this kink site is too long (3.94 Å) to be a hydrogen bond, whereas in CA14, the O2(6′) $\cdots$ O3(5′) distance of 3.40 Å is indicative of a long, weak hydrogen bond. The orientations of the glucose units at the flip sites are marked by arrows, and thick arrows at O4 and C1 atoms at both ends of the flip sites delineate the course of the CA chain. Drawn with the program MOLSCRIPT.[19]

causes a herringbone-type arrangement. Consequently, the crystal structures of CA10 and CA14 combine the two packing motifs already identified in inclusion complexes of the smaller cyclodextrins.[12]

The CHARMM force field was used to minimize the energy of the X-ray structures (with calculated H positions)[13] of CA6 to CA10 and CA14. The solvation energy was calculated using the Poisson-Boltzmann program SOLVATE.[14] The sum of force field and solvation energies per glucose unit is similar for all molecules and ranges from -7.42 kcal mol^{-1} (CA6) to -7.94 kcal mol^{-1} (CA10). The individual energy terms in that sum vary significantly, especially for CA10 and CA14; the Coulombic energy per glucose unit is 2 kcal mol^{-1} higher in CA10 than in CA9, this difference being balanced by the more relaxed torsion angle energies and the better solvation of CA10 and CA14.

All very large CAs may exhibit comparable structures, because in CA14 the band-flip site forms short O2(n) $\cdots$ O3($n-1$) hydrogen bonds to preceding and following *cis* glucose residues. Additional glucose residues in larger CAs may be added at the flip sites to form strings of O2(n) $\cdots$ O3($n-1$) hydrogen-bonded glucose residues in *cis* orientation as in cyclodextrins, and the kink may vary (and even disappear), depending on residual strain energy. The two antiparallel strings may approach each other and associate across the center of the molecule through hydrogen bonds between O2 and O3 hydroxyl groups (see caption of Figure 3B). Since the strings will be twisted as a result of their O2(n) $\cdots$ O3($n-1$) hydrogen bonding, we anticipate that they will form a left-handed antiparallel double helix (Figure 2E) as observed in the crystal structure of (p-nitrophenyl-α-maltohexaoside)$_2 \cdot$ Ba(I$_3$)$_2 \cdot$ 27 H$_2$O.[15]

A recent preliminary note[16] reports the structure of CA10 crystallized from a 1:1 mixture of water and acetonitrile. The crystals are identical to ours and did not form a complex with the organic solvent. This suggests that the inclusion properties of CA10 are different to the cyclodextrins CA6 to CA8, which accommodate acetonitrile.[17] In CA10 and the higher homologues the cavities are so distorted into narrow grooves that probably only guest molecules with geometries that exactly fit these grooves can form inclusion complexes. This could open avenues for very specific host–guest interactions with the larger CA molecules.

The situation could be different with CAs large enough to form an antiparallel double helix (Figure 2E) containing a central, channel-like cavity that may accommodate guest molecules.[15a] This view is supported by the observation[18] that CA50 and larger amyloses show properties similar to linear amylose. They form precipitates from aqueous solutions if higher alcohols or long fatty acids are added and produce blue or brown complexes with iodine (the color depends on size).

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[5] CA mixtures, produced by cyclodextringlycosyltransferase acting on synthetic amylose,[4b] were purified by HPLC and characterized by time-of-flight (TOF) MS. ^{13}C NMR spectra (125.65 MHz) of the CAs were recorded at 2–3 % (w/v) in D$_2$O at 50°C on a JOEL GSX-500 spectrometer.

[6] X-ray data of CA10[7] measured at 20°C with a CAD4 diffractometer on an FR571 rotating anode X-ray generator (Enraf-Nonius) with Ni-filtered Cu$_{K\alpha}$ radiation. Monoclinic, space group $C2$, $a = 29.242(5)$, $b = 9.981(1)$, $c = 19.319(2)$ Å, $\beta = 120.97(2)°$, $V = 4834.7$ Å^3 with half a molecule in the asymmetric unit, composition $(C_6H_{11}O_5)_{10} \cdot 20.7\,H_2O$, $\rho_{calcd} = 1.380$ g cm^{-3}, 3158 unique reflections. Structure determined by direct methods (SIR92) and full-matrix least-squares refinement (SHELXL76). Crystallographic R factor 9.31 % for 2760 data with $F \geq 3\sigma_F$. O6 hydroxyl groups of glucose residues 1–3 and 5 doubly disordered; 17 water positions, some partially (0.3–1.0) occupied.

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Crystal structure analysis of CA14[7] as reported for CA10. Monoclinic, space group $C2$, $a = 36.750(9)$, $b = 10.138(2)$, $c = 21.236(4)$ Å, $\beta = 116.18(2)^\circ$, $V = 7093.5$ Å^3 with half a molecule in the asymmetric unit, composition $(C_6H_{11}O_5)_{14} \cdot 27.3\,H_2O$, $\rho_{calcd} = 1.301$ g cm^{-3}, 4633 unique X-ray reflections. Crystallographic R factor 9.71 % for 3478 data with $F \geq 3\sigma_F$. O6 hydroxyl groups of glucose residues 1, 4, and 6 doubly disordered; 24 water positions, some partially (0.3 – 1.0) occupied. A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *J. Appl. Cryst.* **1994**, *27*, 435; G. M. Sheldrick, SHELXL76 *Program for Crystal Structure Determination*, University of Cambridge, UK, **1976**.

[7] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100 656. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[8] Atom numbering: O2(4) means oxygen atom O2 in glucose residue 4; units with primed labels belong to the second half of the molecule. Disordered O6(n)A is in the preferred $-$*gauche* and O6(n)B in the less common $+$*gauche* orientation.

[9] Definition of ϕ and ψ torsion angles: O5(n)-C1(n)-O4($n-1$)-C4($n-1$) and C1(n)-O4($n-1$)-C4($n-1$)-C3($n-1$); see IUPAC rules: *Eur. J. Biochem.* **1983**, *131*, 5 – 7.

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Combinatorial Approach to the Hydrothermal Synthesis of Zeolites

Duncan E. Akporiaye,* Ivar M. Dahl, Arne Karlsson,* and Rune Wendelbo*

Recent automated combinatorial chemical methods have been developed for systematic production and evaluation of materials in the fields of organic[1] and inorganic[2] chemistry. Until now, however, there have not been any reports of methods capable of dealing with the special conditions used in the synthesis of zeolites, such as temperatures above the normal boiling point of the reaction mixture and under elevated pressures. To address this problem we have developed an autoclave[3] capable of carrying out at least 100 crystallizations under hydrothermal conditions at temperatures up to 200 °C (Figure 1). The simplest most inexpensive design consists of a Teflon block in which 100 reaction chambers are formed by cylindrical holes, having properly designed profiles to accommodate Teflon-coated septa, which seal the chambers when they are "sandwiched" in between two steel plates.

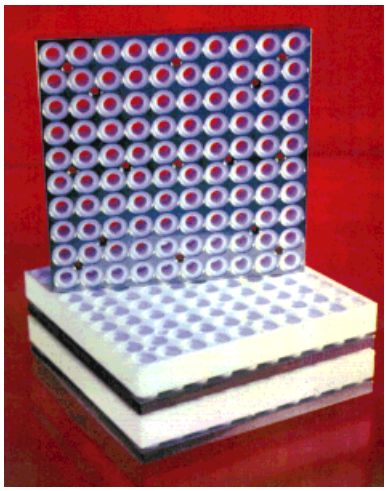

Figure 1. View of the multiautoclave showing the mode of stacking of the Teflon blocks and one of the alternative designs using Teflon inserts.

An important advantage of the system design, which highlights its versatility, is the capability of stacking identical synthesis blocks, allowing an expansion in the third dimension and the parallel synthesis of the order of 1000 combinations in one experiment. The autoclave fits into the compartment of commercial pipette robots for convenient formulation of the synthesis gels. After crystallization, the contents of the autoclave can be washed in situ before further processing. Procedures for a fully automated powder X-ray diffraction analysis of the arrays of materials that are obtained are in progress. To

[*] Dr. D. E. Akporiaye, Dr. A. Karlsson, Dr. R. Wendelbo, I. M. Dahl
Sintef Applied Chemistry
P. O. BOX 124, Blindern, N-0314 Oslo (Norway)
Fax: (+47) 2206-7350
E-mail: Duncan.Akporiaye@chem.sintef.no
Arne.Karlsson@chem.sintef.no
Rune.Wendelbo@chem.sintef.no

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