Variation of a Theme: Crystal Structure with Four Octakis(2,3,6-tri-*O*-methyl)-γ-cyclodextrin Molecules Hydrated Differently by a Total of 19.3 Water[†,‡]

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Abstract: Octakis(2,3,6-tri-*O*-methyl)-γ-cyclodextrin (TRIMEG) crystallized from saturated aqueous solution at 18 °C in the monoclinic space group $P2_1$ with 4 TRIMEG and a total of 19.3 water molecules in the asymmetric unit $[4(C_{72}H_{128}O_{40})]\cdot 19.3H_2O$. The structure was solved by ab initio real/reciprocal space recycling procedure and refinement converged at an *R*-factor of 0.084 for 19 782 data with $F^2 > 2\sigma(F^2)$. The structures of the four TRIMEG molecules are similar with O4 atoms nearly coplanar and pseudo-2-fold rotation axes passing through the centers of the macrocycles. The glucoses are oriented syn except for the diametrically opposed glucoses 1 and 5 which are flipped by ∼180° (anti), their C5−C6−O6−C9 chains being rotated toward the center and closing the cavity from one side yielding bowl-shaped molecules; the O6 atoms of these chains are hydrogen bonded to water molecules located on the pseudo-2-fold axes. The 19.3 water molecules are distributed over 27 sites hydrogen bonded in different patterns to the 4 TRIMEG.

Introduction

The α-, β-, and γ-cyclodextrins (CDs) are a family of cycloamyloses composed of 6, 7, and 8 α(1 → 4) linked D-glucoses, respectively.[1] As shown by a large number of X-ray studies,[2] they are annular, cone-shaped molecules with all glucoses in the $^4C_1$-chair conformation and oriented syn, i.e., their O6 hydroxy groups are on the narrow side of the cone and their O2, O3 hydroxy groups on the wide side. The latter are systematically connected by intramolecular, interglucose O2-(*n*)···O3(*n* − 1) hydrogen bonds stabilizing the structure of the macrocycle. The CDs are well-known for the inclusion of molecules which are small enough to fit into their central cavity,[3] a property not only of academic but also of commercial interest.

If the glucoses of the CDs are dimethylated at their O2 and O6 or trimethylated at their O2, O3, and O6 hydroxy groups (Figure 1), the inclusion properties improve for some of the guest molecules.[4] More interestingly, their solubility coefficient in water becomes negative, i.e., they are better soluble in cold than in hot water, where they precipitate or crystallize.[5]

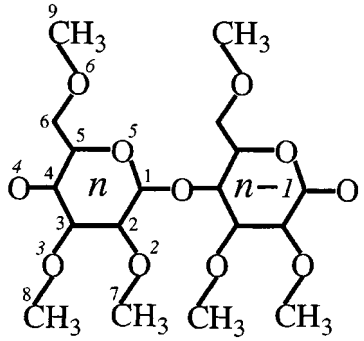

Figure 1. Atomic numbering scheme showing two trimethylated glucoses in syn orientation.

For further investigation of this property, methylated CDs have been crystallized from hot and cold water at 60−80 °C and at 4−18 °C, respectively, and were subjected to X-ray analyses. The crystals which could be obtained from hot water are anhydrous hexakis(2,6-di-*O*-methyl)-α-CD (DIMEA),[6,7] anhydrous heptakis(2,6-di-*O*-methyl)-β-CD (DIMEB),[8] anhydrous hexakis(2,3,6-tri-*O*-methyl)-α-CD (TRIMEA),[9] heptakis-(2,3,6-tri-*O*-methyl)-β-CD (TRIMEB)·H_2O,[10] and octakis(2,3,6-

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† Topography of cyclodextrin inclusion complexes, part 45. For part 44, see Aree, T.; Saenger, W.; Leibnitz, P.; Hoier, H. *Carbohydr. Res.*, in press.

‡ Data have been deposited with the Cambridge Crystallographic Data Center and may be obtained, on request, from the director, Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2, IEZ, U.K.

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tri-*O*-methyl)-γ-CD (TRIMEG)·2H_2O.[11] In these crystals, the methylated CDs adopt a form reminiscent of a bowl rather than a truncated, open cone because two or three of the O6−CH_3 groups are rotated "inward" and close the molecular cavity. The cavities are only filled by O6−CH_3 groups of symmetry-related CD ("self-inclusion") and not by water molecules which occupy the intermolecular space between the CD molecules.

If methylated CDs are crystallized from cold water (18 °C), the structures of the macrocycles are different because they adopt the shape of an open cone and the molecular cavity hosts one water molecule—other waters may be located in interstices between the methylated CDs as in DIMEA·H_2O[6] and DIMEB·2H_2O.[12]

In this paper, we describe the structure of TRIMEG crystallized from an aqueous solution at 18 °C. The asymmetric unit of the crystal contains four independent TRIMEG molecules which are hydrated differently with a total of 19.3 water molecules distributed over 27 sites.

Experimental Section

1. Crystallization and X-ray Diffraction Experiments. TRIMEG purchased from Cyclolab (Budapest/Hungary) was used without further purification. A saturated aqueous solution was prepared at 0 °C and kept at 4 °C for about 1 month, but no crystals were formed. However, when the vials containing these solutions were transferred to a microscope at 18 °C for visual inspection, large prisms 3 × 3 × 4 mm^3 in size crystallized spontaneously within 30 min. A crystal of 0.3 × 0.7 × 1.0 mm^3 was sealed together with a drop of mother liquor in a quartz capillary and used for X-ray data collection performed at room temperature with a Bruker AX3 SMART diffractometer equipped with a CCD area detector using graphite-monochromated Mo Kα radiation, $\lambda = 0.710\ 73$ Å. The 77 093 data were collected in the θ-range 1.42−23.78° (0.88 Å resolution). Data reduction and semiempirical absorption correction from ψ-scans were carried out using the programs SAINT and SHELXTL, to yield 19 782 reflections with $F^2 > 2\sigma(F^2)$. Space group is monoclinic $P2_1$ according to systematic absences *0k*0, crystal unit cell dimensions are $a = 29.872(3)$, $b = 18.018(2)$, $c = 33.170(3)$ Å, $\beta = 98.15(1)°$, and there are four TRIMEG molecules and a total of 19.3 water molecules in the asymmetric unit (see Table 1).

2. Structure Solution and Refinement. Several attempts to determine the structure failed. In molecular replacement, the model for TRIMEG taken from ref 11 was too different from TRIMEG in this crystal structure, and in direct methods, the success rate falls off above 150 atoms (four TRIMEGs have 448 C and O atoms). The structure was finally determined by a novel "ab initio" real/reciprocal space recycling procedure[13] implemented in SHELXD. It is inspired by, but different in detail, from the "shake and bake" method.[14] It provided all TRIMEG C and O atoms except for some disordered methyl groups and several of the water O atoms. The remaining atoms were located from difference Fourier maps. Due to the large structure (467 non-hydrogen atoms) and the relatively low data/parameter ratio, refinement strategies were similar to those for macromolecular crystal structures utilizing distance and displacement parameter restraints and block-matrix conjugate gradient least squares on F^2 as implemented in SHELXL97.[15] The option SADI was employed to improve the geometry of each of the 32 glucose units by restraining the corresponding 1,2- and 1,3-distances to be equal. Water sites and disordered methyl groups were located from difference electron density maps, C−H hydrogen atoms were placed in calculated positions—those of water could not be determined. All non-hydrogen atoms were treated anisotropically, refinement against 4341 parameters converged at $R = 0.084$ for 19782 data with $F^2 > 2\sigma(F^2)$.

Table 1. Crystallographic Data; Data Collection, Solution and Refinement for [4TRIMEG]·19.3H_2O

chemical formula	$[4(C_9H_{16}O_5)_8]$·19.3H_2O
formula weight	6843.66
crystal habit, color	plate, colorless
crystal size (mm^3)	0.3 × 0.7 × 1.0
crystal system	monoclinic
space group	$P2_1$
unit cell dimensions	
a (Å)	29.872(3)
b (Å)	18.018(2)
c (Å)	33.170(3)
β (deg)	98.15(1)
vol ($Å^3$)	17 673(4)
Z	2
D_{calc} (g cm^{-1})	1.286
μ (mm^{-1})	0.107
F(000)	7349
diffractometer	Bruker, CCD
wavelength (Å)	Mo Kα; 0.710 73
temp (°C)	room temperature
θ range for data collection (deg)	1.42−23.78 (0.88 Å resolution)
measured reflections	77 093
unique reflections	26 821 ($R_{int} = 0.0317$)
unique reflections $[F^2 > 2\sigma(F^2)]$	19 782
index ranges	$-33 < h < 32$, $0 < k < 20$, $0 < l < 37$
structure solution	ab initio method (SHELXD)
refinement method	distance and displacement parameter restraints and block matrix conjugate-gradient least-squares against F^2
weighting scheme	$w = [S^2(F_o^2) + (0.0915P)^2 + 17.5934P]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$
data/parameters	26 821/4341
R $[F^2 > 2\sigma(F^2)]$	$R^a = 0.084$, $wR^b = 0.203$
R (all data)	$R = 0.110$, $wR = 0.222$
goodness of fit	1.062
highest peak and deepest hole (e $Å^{-3}$)	0.65, −0.39

[a] $R = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $wR = \sum\{w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2\}^{1/2}$.

As shown in Figures 2 and 3a and Table 2, parts b and c, several of the methyl and O6−CH_3 groups are 2-fold disordered. These are O63−C93 and O64−C94 in TRIMEG 2; C76, C96, and O68 in TRIMEG 4. The 19.3 water molecules are distributed over 27 sites with occupation factors in the range 0.18−1.00, see point 4, below.

Results and Discussion

In the following, the atoms in the four TRIMEG molecules are designated first with their chemical number (see Figure 1), second the number of the glucose unit (1−8), and third the TRIMEG molecule (1−4), Figure 2. For example, O37_4 denotes atom O3 in glucose 7 of TRIMEG 4. The methyl carbon atoms on O2, O3, and O6 are designated as C7, C8, and C9, respectively. In addition, the disordered atoms are labeled with the letters A and B.

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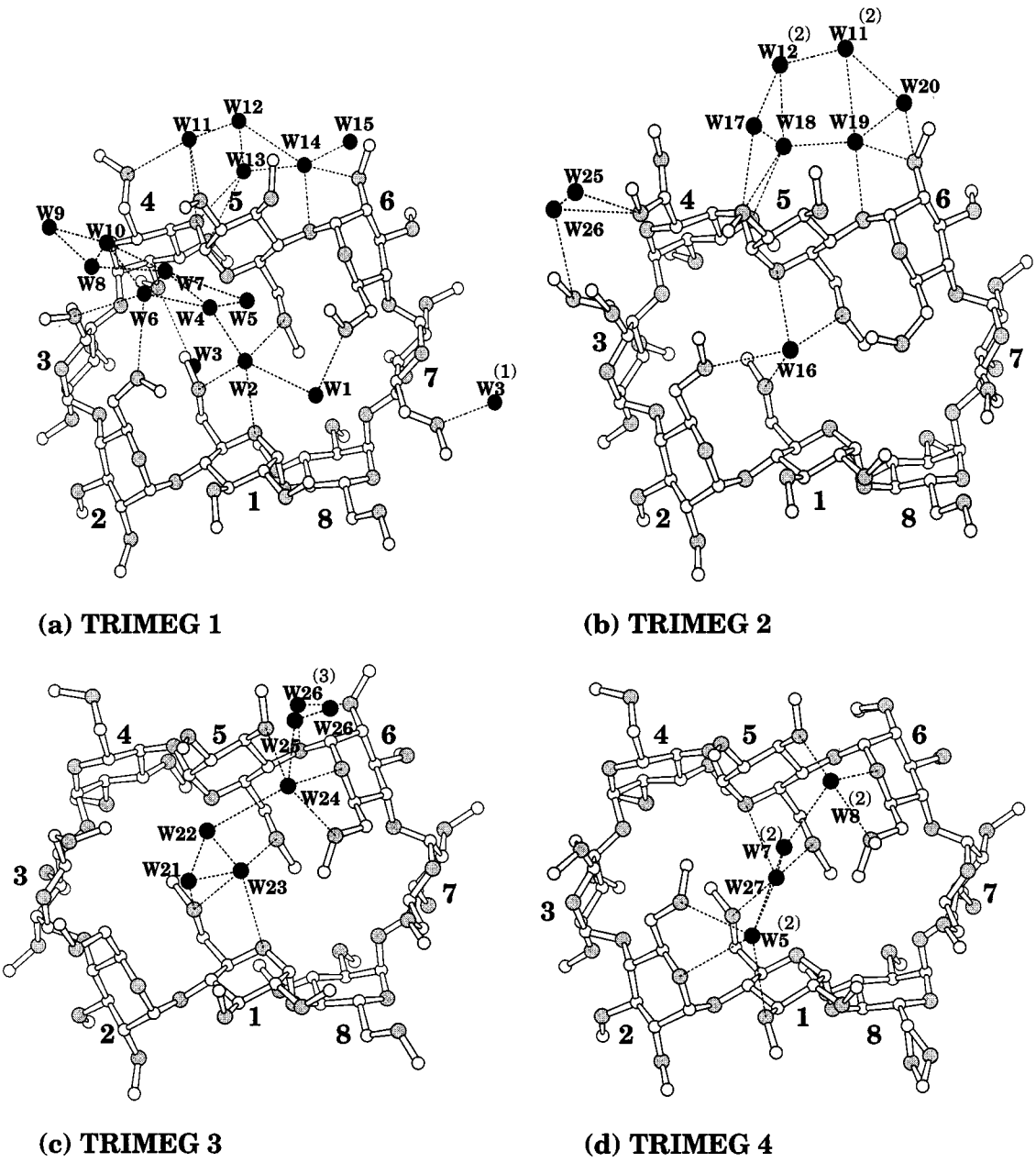

Figure 2. Structures of individual TRIMEG molecules with different hydration patterns in [4TRIMEG]·19.3H_2O, (a) TRIMEG 1, (b) TRIMEG 2, (c) TRIMEG 3, and (d) TRIMEG 4. The unfilled, shaded, and filled circles are C, O, and O_w, respectively. The dashed lines indicate possible hydrogen bonds. Water sites with superscripts (1), (2), and (3) are in symmetry equivalent positions: $-x$, $0.5 + y$, $1 - z$; $1 + x$, y, z; and $1 - x$, $-0.5 + y$, $1 - z$; respectively (see also Figure 5 for structural details of hydrogen bond networks of water sites). Drawn with the program MOLSCRIPT.[22]

1. The Conformations of the Four TRIMEG Molecules Are Almost Identical with Pseudo-2-Fold Rotation Symmetries. All four TRIMEG molecules in the asymmetric unit are similar in shape and can be superimposed with rms deviations in the range 0.34−0.52 Å (omitting O6 and all CH_3 groups), Figure 3a. The TRIMEG molecules have an elliptical rather than the "round" shape known for the native CDs. The long axis of the ellipse passes through glucoses G3 and G7 and the short axis through G1 and G5 (see also Figure 2). The molecules have local pseudo-2-fold rotation axes passing through water molecules located within the molecular cavities. The water molecules are hydrogen bonded to O61 and O65 which belong to C5−C6−O6−C9 chains rotated "inward" to close the molecular cavities from one side so that the TRIMEG molecules adopt the bowl-shape (Figure 4) characteristic of methylated CDs crystallized from hot water.[6−11]

Glucoses G2, G3, and G4 and G6, G7, and G8 are in syn orientation, i.e., their O2−CH_3, O3−CH_3 groups are on one side of the macrocycle and O6−CH_3 on the other as shown in Figure 1. By contrast, G1 and G5 are flipped by ∼180° into an anti orientation so that atoms O61 and O65, which are rotated toward the cavity, can accept hydrogen bonds from the water located on the pseudo-2-fold rotation axis. Individual geometrical data are provided in Table 2. All glucose residues adopt the $^4C_1$-chair conformation, with some slight distortions as indicated by their puckering parameters,[16] Q within 0.49−0.58 Å and θ within 2−17°. The deviations of individual O4 atoms from their mean plane do not exceed 1.34 Å, the tilt angles of glucoses with respect to this plane are in the range 12.9−57.2°, O4(n)···O4(n − l) distances are within 4.04−4.55 Å; O4(n + l)···O4(n)···O4(n − l) angles are in the range 119.5−145.5°. The largest variations are found with the torsion angles ϕ, ψ

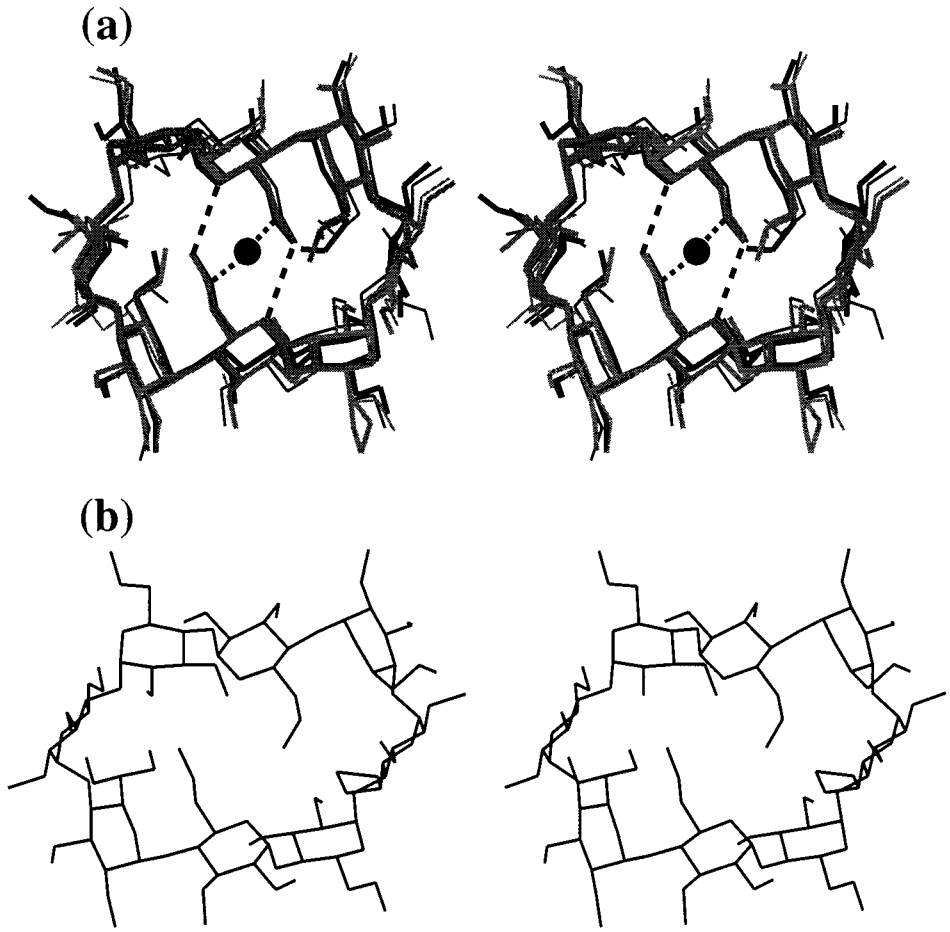

Figure 3. (a) Stereo plot of the overlay of four TRIMEG molecules in [4TRIMEG]·19.3H_2O. The thin and thick, black and gray lines are TRIMEG 1, TRIMEG 2, TRIMEG 3, and TRIMEG 4, respectively. The filled circle indicates the common water molecule located on the pseudo-2-fold rotation axes of TRIMEG molecules. Dotted and dashed lines show systematic hydrogen bonds of O_w−H···O61, O_w−H···O65, and C91−H···O55, C95−H···O51, respectively. (b) Stereo plot of TRIMEG molecular structure in TRIMEG·2H_2O[11] (two water molecules located ouside the cavity not shown).

describing the orientation of adjacent glucoses with respect to their glycosidic link (defined as ϕ, O5(n)−C1(n)−O4(n − l)−C4(n − l); ψ, C1(n)−O4(n − l)−C4(n − l)−C3(n − l)),[17]Table 2a. They are in the range commonly observed for syn-oriented glucoses (ϕ, 53−100°; ψ, 70−122°) and differ for the two anti-oriented glucoses G1 and G5 (ϕ, 81−96°; ψ, −60 to −72°).

2. The Orientation of the Methyl Groups at O2 and O3 Is Restricted. The orientation of the methyl groups relative to the associated glucose is best described for the secondary O2−CH_3, O3−CH_3 by torsion angles C1−C2−O2−C7 and C2−C3−O3−C8 and for the primary O6−CH_3 by the two torsion angles O5−C5−C6−O6 and C5−C6−O6−C9. Torsion angles C1−C2−O2−C7 are in the +gauche range, 74.8−120.4° except for G6 of TRIMEG 4 where C7 is 2-fold disordered and the torsion angle is −gauche (−65.3°; −60.3°). This means (except for G6 of TRIMEG 4) that C7 is on the same side of the plane described by glucose atoms C2, C3, C5, and O5 as atom C6 (endo). By contrast, torsion angles C2−C3−O3−C8 are −gauche (−74.3 to −113.1°) so that C8 is on the opposite side of this plane (exo), except for G1 of TRIMEG 3 where this torsion angle is +gauche (59.5°).

Torsion angles describing the orientations of O6 and C9 are more variable (Table 2, parts b and c) except for glucoses G1 and G5 which are oriented anti. For these glucoses, O5−C5−C6−O6 is −gauche (−61.2 to −68.3°) and C5−C6−O6−C9 is trans (169.8−179.3°) and so narrowly confined that the O61 and O65 atoms are in the correct position to form hydrogen bonds to the water molecules located on the pseudo-2-fold axes (Figures 2 and 3a). The corresponding torsion angles of the other glucoses are much more variable, in +gauche and −gauche for O5−C5−C6−O6 and, in addition, trans for C5−C6−O6−C9.

3. Stabilization of Macromolecular Conformation by Hydrogen Bonds. The conformation and pseudo-2-fold symmetry of the four TRIMEG molecules is stabilized by the anti orientation of the diametrically opposed glucoses G1 and G5 (Figures 2 and 3a). Their O6 atoms, O61 and O65, accept hydrogen bonds (in the O···O distance range 2.738−3.178 Å) from the water molecules located on the respective pseudo-2-fold axes, and their C9 methyl groups are engaged in C9−H···O5 interactions, i.e., C91−H···O55 and C95−H···O51 (in the C···O distance range 3.167−3.561 Å), Figures 2 and 3a. In addition, this water site is in close C−H···O type contact to both or only one C9 methyl groups of glucoses G2 and G6 in TRIMEG molecules 2−4. In molecule 1, however, such interactions are not found due to the large number of water positions which appear to impede these contacts. Furthermore, a number of C−H···O hydrogen bonds contribute to crystal structure stabilization as frequently observed in other carbohydrate structures[18] (not shown).

4. Three Water Clusters. The 19.3 water molecules in the asymmetric unit are distributed over 27 sites. These sites are hydrogen bonded to form three clusters which are separated from each other (see Figures 2 and 5). One of the clusters contains 10 water sites bonded to TRIMEG 1 and 4, one cluster,

Table 2. Geometrical Parameters of [4TRIMEG]·19.3 H_2O Crystal Structure (Angles in Degrees)

residue	TRIMEG 1	TRIMEG 2	TRIMEG 3	TRIMEG 4
		(a) Torsion Angles ϕ and ψ[a]		
1	87.3(7), −64.3(8)	92.1(8), −60.4(9)	88.2(9), −64.9(11)	95.8(9), −59.5(12)
2	55.7(9), 74.6(9)	53.6(10), 71.7(10)	57.4(10), 69.8(10)	53.3(9), 79.8(10)
3	90.5(7), 110.1(7)	88.0(8), 103.4(8)	96.6(8), 122.4(8)	86.9(7), 99.4(7)
4	92.9(7), 109.1(7)	94.9(8), 120.6(7)	93.2(7), 112.1(7)	99.5(7), 115.7(7)
5	81.4(8), −71.9(9)	85.6(9), −65.8(10)	87.7(8), −65.6(9)	86.3(8), −67.4(9)
6	59.4(9), 79.8(9)	51.4(11), 80.0(10)	48.0(11), 74.5(10)	51.2(12), 70.2(12)
7	92.5(8), 114.5(8)	87.6(9), 106.5(8)	95.3(9), 117.9(9)	97.6(10), 121.7(10)
8	92.9(8), 103.3(7)	97.0(8), 109.9(8)	91.5(9), 99.2(9)	94.4(9), 93.5(10)
		(b) Torsion Angles O5−C5−C6−O6		
1	−62.6(8)	−64.1(9)	−68.2(11)	−61.2(10)
2	83.4(10)	78.9(12)	−69.3(11)	66.6(13)
3	71.6(10)	−59.1(17),[b] 69.5(15)[b]	73.9(10)	67.6(10)
4	−76.9(10)	−66.3(14),[c] 78.2(21)[c]	−69.3(10)	−73.5(10)
5	−64.9(9)	−66.4(10)	−66.3(9)	−68.3(10)
6	75.8(11)	95.8(13)	65.0(13)	67.7(15)
7	−61.1(9)	67.7(15)	78.8(11)	77.7(16)
8	71.0(11)	67.4(12)	70.3(14)	−38.1(22),[d] −72.7(17)[d]
		(c) Torsion Angles C5−C6−O6−C9		
1	169.8(8)	172.6(9)	176.4(9)	174.4(9)
2	−70.9(22)	−175.6(29)	−175.2(13)	112.9(24)
3	−117.2(17)	165.2(35),[e] −172.6(32)[e]	84.1(12)	−89.5(11)
4	106.5(17)	−172.3(18),[f] 171.9(45)[f]	92.7(12)	86.4(12)
5	174.2(8)	179.3(9)	178.5(8)	176.9(9)
6	−83.4(17)	−81.2(24)	111.6(22)	103.2(27),[g] −144.5(20)[g]
7	179.7(10)	157.3(19)	−171.6(14)	−169.3(19)
8	−177.4(12)	177.9(16)	−150.6(20)	−143.6(21),[h] 173.8(16)[h]

[a] Torsion angles ϕ and ψ at glycosidic O4, defined as O5(n)−C1(n)−O4(n−1)−C4(n−1) and C1(n)−O4(n−1)−C4(n−1)−C3(n−1), respectively.[16] [b] Values for disordered O63_2 with occupancies 0.45 and 0.55 for sites A and B, respectively. [c] Values for disordered O64_2 with occupancies 0.75 and 0.25 for sites A and B, respectively. [d] Values for disordered O68_4 with equal occupancies 0.50 for both sites A and B. [e] Values for disordered O63−C93_2 with occupancies 0.45 and 0.55 for sites A and B, respectively. [f] Values for disordered O64−C94_2 with occupancies 0.75 and 0.25 for sites A and B, respectively. [g] Values for disordered C96_4 with occupancies 0.53 and 0.47 for sites A and B, respectively. [h] Values for disordered O68_4 with equal occupancies 0.5 for both sites A and B.

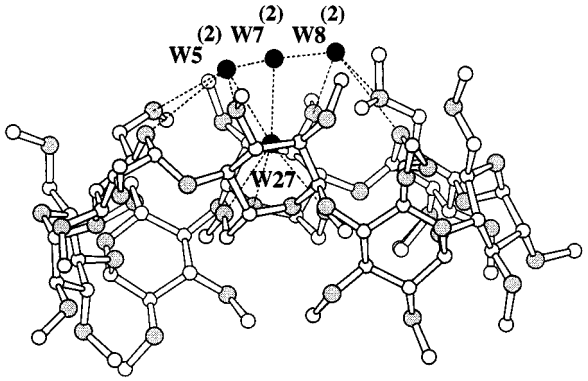

Figure 4. Side view of TRIMEG 4 to show the bowl-shaped structure of TRIMEG molecules in [4TRIMEG]·19.3H_2O. Dashed lines indicate possible hydrogen bonds. Water sites with superscripts (2) are in symmetry equivalent positions: 1 + x, y, z. The two glucoses in the center are the anti-oriented G1 and G5.

of 9 sites, binds to TRIMEG 1 and 2, and the smallest cluster, with 6 sites, is bonded to TRIMEG 2 and 3. One individual water site, W3, binds to TRIMEG 1, and one, W16, to TRIMEG 2. The water sites are all in hydrogen−bonding distance except for some water sites with low occupancies for which O···O distances are in the range 2.027−2.436 Å, i.e., these water sites cannot be occupied simultaneously (Figure 5). These short distances are W8−W10 and W9−W10 in panel a, W12−W13 and W13−W14 in panel b, and W21−W22, W21−W23, W25−W26 in panel c.

5. TRIMEG Molecules Are Differently Hydrated, with Some Common Patterns. In all four TRIMEG structures, one water molecule is located on the pseudo-2-fold axis as an integral part of their structures (TRIMEG 1, W2; TRIMEG 2, W16; TRIMEG 3, W23; TRIMEG 4, W27). The TRIMEG molecules are further hydrated, with some recurrent patterns (Figure 2): (i) in TRIMEG 3 and 4, W24 and W8, respectively, form hydrogen bonds to glucoses G5 and G6, involving O35, O56, and O66; (ii) one water site bridges O26 and O45 in TRIMEG 1, 2, and 3 (water W14, W19, and W26); (iii) one water site bridges O6 of adjacent syn-oriented glucoses in TRIMEG 1 and 2; W6 bridges O62 and O63 in TRIMEG 1; W26 bridges O63A and O64B in TRIMEG 2; and (iv) two water molecules bridge O4 of anti-oriented glucoses G4 and G5 in TRIMEG 1 and 2; O44···W13···W14···O45 and O44···W18···W19···O45.

Conclusions

The four independent TRIMEG molecules have a similar elliptical shape with pseudo-2-fold rotational symmetry. This conformation appears to be imposed and stabilized by the ∼180° flipping (anti-orientation) of the two diametrically opposed glucose units G1 and G5 whose C5−C6−O6−C9 chains are rotated toward the molecular cavity and have nearly identical conformations. This permits O61 and O65 to form hydrogen bonds to a water molecule (O_w) located on the pseudo-2-fold axis, and in addition, there are systematic C−H···O interactions of type C91−H···O55 and C95−H···O51. These interactions close the molecular cavity from one side so that the TRIMEG molecules have a bowl-shaped form (Figure 4) similar to other methylated CDs crystallized from *hot* water.[6−11] The other side of the cavity is not closed but narrowed by "inward" rotation of O6−C9 groups of glucoses G2 and G6, a conformation stabilized by C9−H···O_w interactions to the water molecule O_w located on the pseudo-2-fold axis.

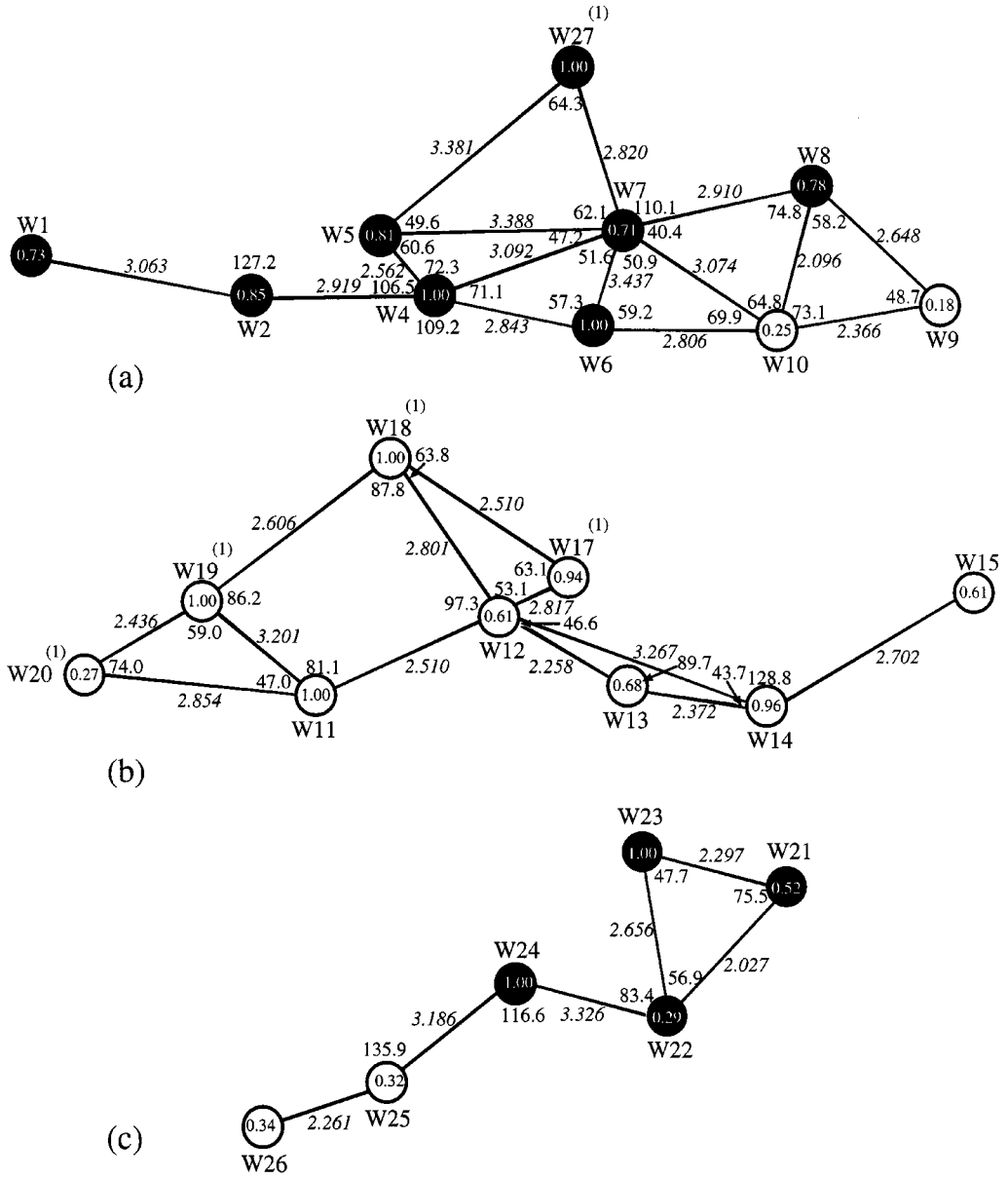

Figure 5. The three distinct water clusters in [4TRIMEG]·19.3H_2O, (a) 10 water sites bind to TRIMEG 1 and 4, (b) nine water sites bind to TRIMEG 1 and 2, and (c) six water sites bind to TRIMEG 2 and 3 (see also Figures 2). The filled and unfilled circles describe the water sites which are inside and outside the TRIMEG cavities, respectively. The numbers written in the circles indicate the occupancies of water sites. Water sites with superscripts (1) indicate the symmetry equivalent position: $-1 + x, y, z$. Given distances are in angstroms and angles in degrees.

The hydration schemes of the four TRIMEG molecules differ from each other, but some recurrent patterns are observed. Even O4 is engaged as a hydrogen bond acceptor, a feature which does not occur in the native CDs. The reason is that the hydrogen bond accepting O4 are located on both sides (O44, O45) of the flipped glucose G5; thus, the O4 atoms become exposed and facilitate hydrogen bonding, as shown with TRIMEG 1 and 2.

On comparison of the structures of TRIMEG crystallized from aqueous solutions at 18 °C and at 80 °C, one striking difference can be observed: at 80 °C, TRIMEG crystallizes as dihydrate[11] with both water molecules located in voids between symmetry-related molecules and *not* in the TRIMEG cavity. The molecule is bowl-shaped and closed on one side by "inward" rotation of O6−CH_3 groups of two diametrically opposed glucoses in anti orientation, similar as described in the present structure. However, the rms deviations of superpositions between the four TRIMEG in the present structure and TRIMEG in dihydrated form are in the range 0.48−0.88 Å and show that they are different in detail, explaining why TRIMEG taken from the dihydrate crystal structure was not successful in molecular replacement. The difference in structure is due to lack of hydration water molecules in TRIMEG dihydrate,[11] particularly the one at the pseudo-2-fold rotation axis of TRIMEG hydrogen bonding the two anti-oriented glucoses (see Figure 3).

The flipping of two glucoses into anti orientation appears to be a molecular property of TRIMEG. It is probably associated with the inability to form O2(n)···O3(n − 1) hydrogen bonds which contribute to the stability of the syn orientation of glucoses if O2−H and/or O3−H hydroxyl groups are not methylated. For unmodified CDs, flipping of glucoses was only observed for the larger ϵ-CD,[19] ι-CD,[19] and a cycloamylose consisting of 26 glucose units[20] where it served to reduce steric strain. In these larger cycloamyloses, however, the flip does not only involve one individual glucose as in TRIMEG. The appended glucoses are also flipped as they are all connected by O2(n)···O3(n − 1) hydrogen bonds, and this is why this flip was termed "band flip" (like cutting a band, rotating one end by 180° and gluing the two ends together).[19,20]

The negative solubility coefficient of TRIMEG may be correlated with molecular hydration rather than with molecular conformation. On the basis of the crystal structure of [4TRIMEG]·$19.3H_2O$, where well-defined hydration patterns are observed, we assume that other methylated CDs are hydrated in a similar way at low temperature, which may explain their high solubility. When the temperature is increased, water molecules become more mobile, the ordered hydration breaks down, and the hydrophobic, methylated CD molecules aggregate and crystallize. Another scenario could involve the increased rotational mobility of the methyl groups (especially the C5−C6−O6−C9 chains) which would interfere with hydration especially at elevated temperatures. From the crystallographic studies alone, we cannot determine the pathway which leads from high solubility in cold water to low solubility at higher temperature. The methylated CDs may serve as a very good model system to study the hydrophobic effect that has given rise to much controversy during the past 60 years.[21]

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Supporting Information Available: Table of crystallographic data, table of atomic coordinates and displacement factors, and a figure of thermal ellipsoid plots (40%) of TRIMEGs 1−4. This material is available free of charge via the Internet at http://pubs.acs.org.

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