Effect of Peracylation of β-Cyclodextrin on the Molecular Structure and on the Formation of Inclusion Complexes: An X-ray Study

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Abstract: The molecular structures of peracylated β-cyclodextrins (CDs)—heptakis(2,3,6-tri-*O*-acetyl)-β-CD (TA), heptakis(2,3,6-tri-*O*-propanoyl)-β-CD (TP), and heptakis(2,3,6-tri-*O*-butanoyl)-β-CD (TB)—have been determined by single crystal X-ray structure analysis. Due to the lack of O2···O3′ hydrogen bonds between adjacent glucose units of the peracylated CDs, the macrocycles are elliptically distorted into nonplanar boat-shaped structures. The glucose units are tilted with respect to the O4 plane to relieve steric hindrance between adjacent acyl chains. In TB, all glucose units adopt the common $^4C_1$-chair conformation and one butanoyl chain intramolecularly penetrates the cavity, whereas, in TA and TP, one glucose unit each occurs in $^OS_2$-skew-boat conformation and one acyl chain closes the O6 side like a lid. In each of the three homologous molecules the intramolecular self-inclusion and lidlike orientation of acyl chains forces the associated O5−C5−C6−O6 torsion angle into a *trans*-conformation never observed before for unsubstituted CD; the inclusion behavior of TA, TP, and TB in solution has been studied by circular dichroism spectroscopy with the drug molsidomine and several organic compounds. No inclusion complexes are formed, which is attributed to the intramolecular closure of the molecular cavity by one of the acyl chains.

Introduction

Degradation of the amylose fraction of starch by glucosyltransferase yields cyclic oligosaccharides consisting of six, seven, or eight α(1−4)-linked D-glucose residues, known as α-, β-, and γ-cyclodextrins (CDs). They resemble truncated, hollow cones in which all glucoses adopt the commonly observed $^4C_1$-chair conformation and are oriented syn; secondary hydroxyl groups (O2, O3) are on the wide side of the cone and form intramolecular, interglucose O2···O3′ hydrogen bonds stabilizing the conformation of the CD macrocycles,[1−4] and the primary hydroxyl groups (O6) are on the narrower side of the cone. The hydroxyl groups render the CDs hydrophilic outside; in contrast, their central cavities are hydrophobic because they are coated with hydrogen atoms from the C3−H and C5−H methine groups, the C6−H_2 methylene groups, and the glycosidic oxygens (O4). The primary O6−H hydroxyl groups may rotate about the C5−C6 bonds with torsion angles (χ^5(O5−C5−C6−O6)) preferentially in (−)gauche conformation, so that O6 point "away" from the center of the cavity; in (+)gauche, O6 points to the center of the cavity and is frequently found hydrogen bonded to guest molecules. Due to adverse steric interactions, the "flipped" trans conformation has never been observed so far.

One of the most important features of the CDs is the ability to form inclusion complexes with a large variety of guest molecules and ions that have a suitable size and shape to be fully or partially accommodated in their central cavities.[1,5−9] This feature is utilized in food and pharmaceutical industries[10] to encapsulate compounds that are sensitive to the environment, have a low solubility in water, or are volatile and have more favorable properties as CD inclusion complexes.

Numerous mono-, di-, and trisubstituted CDs have been synthesized to improve the inclusion selectivity of the host or to introduce catalytic activity. Modification of the hydroxyl groups by hydroxyalkyl, acyl, or longer alkyl groups decreases the solubility of the β-CDs, but their solubility may increase again in the presence of guest molecules.[11] The complex forming and physical properties of peracylated CDs were recently

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(1) Saenger, W. In *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Academic Press: London, 1984; Vol. 2, pp 231−260.
(2) Czugler, M.; Eckle, E.; Stezowski, J. J. *J. Chem. Soc., Chem. Commun*. **1981**, 1291−1292.
(3) Harata, K. *Chem. Lett*. **1985**, 2057−2060.
(4) Mentzafos, D.; Mavridis, I. M.; Le Bas, G.; Tsoucaris, G. *Acta Crystallogr*. **1991**, *B47*, 746−757.
(5) Szejtli, J. *Cyclodextrin Technology*; Kluwer: Dordrecht, The Netherlands, 1988.
(6) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: Berlin, 1978.
(7) Uekama, K.; Irie, T. In *Cyclodextrins and Their Industrial Uses*; Duchêne, D., Ed.; Editions de Santé: Paris, 1987; 395−439.
(8) Saenger, W. *Angew. Chem., Int. Ed. Engl*. **1980**, 19, 344−362.
(9) Harata, K. *Angew. Chem., Int. Ed. Engl*. **1991**, *5*, 311−344.
(10) Frömming, K. H.; Szejtli, J. *Cyclodextrin in Pharmacy*; Kluwer: Dordrecht, The Netherlands, 1994.
(11) Lindberg, B.; Lindberg, J.; Pitha, J.; Rao, C. T.; Harata, K. *Carbohydr. Res*. **1991**, *222*, 113−116.

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Table 1. Crystallographic Data: Data Collection, Solution, and Refinement

	TA-β-CD	TP-β-CD	TB-β-CD
empirical formula	$C_{84}H_{112}O_{56}\cdot CH_4O$	$C_{105}H_{154}O_{56}$	$C_{126}H_{196}O_{56}$
fw	2045.8	2312.3	2606.8
cryst size (mm^3)	0.2 × 0.3 × 0.4	1.0 × 0.6 × 0.3	0.7 × 0.4 × 0.3
cryst syst	monoclinic	monoclinic	orthorhombic
space group	$P2_1$	$P2_1$	$P2_12_12_1$
unit cell dimens			
a (Å)	11.567(8)	12.562(8)	17.631(4)
b (Å)	21.105(9)	21.695(12)	27.254(5)
c (Å)	20.300(15)	21.451(10)	28.616(6)
β(deg)	93.584(7)	93.977(13)	
vol ($Å^3$)	4946(5)	5832(5)	13 750(5)
Z	2	2	4
D_{calc} (g cm^{-1})	1.374	1.317	1.259
m (mm^{-1})	0.117	0.107	0.099
F(000)	2156	2464	5600
diffractometer		Siemens, CCD	
wavelength (Å)		Mo Kα, 0.710 73	
temp (°C)		−133(2)	
measd reflecns:	53 213	83 964	206 120
unique reflecns:			
−all data	7339	12 346	12 367
>4s(*F*)	6231	10 051	10 508
max. resoln (Å)	0.9	0.89	0.9
struct soln		ab initio method (SHELXD)	
refinemt method		SHELXL97	
no. params	1279	1480	1717
R_{sym}[a] (%)	0.0404	0.0304	0.0433
R_w[b] [$F^2 > 2s(F^2)$]	0.0739	0.0744	0.1423
Rc [$F^2 > 2s(F^2)$]	0.0333	0.0337	0.0556
goodness of fit	0.982	0.919	1.057
highest peak and deepest hole (e $Å^{-3}$)	0.2/−0.3	0.29/−0.2	0.63/−0.48

[a] $R_{sym} = \Sigma|I - \langle I\rangle|/\Sigma I$. [b] $R_w = \Sigma\{w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2\}^{1/2}$. [c] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.

investigated for the utilization as slow-release carriers of water-soluble drugs such as molsidomine (a peripheral vasodilator).[12−14] In these studies, complexes of molsidomine with peracylated β-CDs were prepared by the kneading method using 1:1 molar host:guest ratios and ethanol as solvent and characterized by differential scanning calorimetry (DSC), suggesting that molsidomine and perbutanoylated-β-CD form a binary solid dispersion with a 2:1 (drug:CD) molar ratio.[13] Release of molsidomine was markedly retarded by complexation with peracylated β-CDs, and retardation increased with increasing order of hydrophobicity of the acyl groups.

Persubstitution of CDs may lead to structural modifications, associated with alteration of the physical properties discussed above. For instance full methylation causes remarkable distortion of the CD macrocycle, since the typical intramolecular O2···O3′ hydrogen bond pattern cannot be formed. This results in conformational flexibility of the macrocycle to such an extent that glucoses may even flip from syn to anti orientation.[15] Rotation of O6−CH_3 groups to the center of the cavity closes it on one side, leading to a bowl shape of the molecule rather than the open cone.[16] In addition, the conformation of individual glucose units may also be affected. In the crystal structure of permethyl-β-CD·H_2O, one of the trimethylglucose units is converted from $^4C_1$ chair to $^1C_4$ conformation,[15] and in the complex of permethyl-β-CD with *m*-iodophenol, one trimethylglucose unit adopts a $^oS_2$ skew-boat conformation.[17,18]

The main intention of the present paper was to crystallize three peracylated β-CDs: TA (heptakis(2,3,6-tri-*O*-acetyl)-β-CD), TP (heptakis(2,3,6-tri-*O*-propanoyl)-β-CD), and TB (heptakis(2,3,6-tri-*O*-butanoyl)-β-CD) with and without guest molecules and to determine their structures by X-ray diffraction methods. In addition, we investigated their complexing ability in solution with various organic compounds (2,7-dihydroxynaphthalene, 4-iodophenole, hydroquinone, and molsidomine), using circular dichroism spectroscopy.[19,20]

Experimental Section

(1) Crystallization and X-ray Diffraction Experiments. Peracylated-β-CDs were prepared using published procedures.[12−14] Colorless crystals of TA·methanol and of the anhydrates of TP and TB were obtained at 18 °C by slow evaporation of saturated solutions prepared with MeOH/H_2O (8:2 (v/v)); the addition of MeOH was necessary as TA, TP, and TB are nearly insoluble in pure water. For X-ray diffraction experiments, the crystals were mounted in oil (perfluoropolyether) on cryoloops and intensity data were collected at −133 °C (to reduce the expected high-temperature factors) on a Siemens CCD-diffractometer using graphite monochromated Mo Kα radiation (λ = 0.710 73 Å). The total of 53 213 (TA), 83 964 (TP), and 206 120 (TB) reflections were collected in the θ-ranges of 1.93−23.26° (0.9 Å resolution, TA), 1.90−26.46° (0.89 Å resolution, TP), and 1.61−24.59° (0.9 Å resolution, TB). Semiempirical absorption corrections from ι-scans and data reductions were carried out using the programs SAINT and SHELXL to yield 7339 (TA), 12 346 (TP), and 12 367 (TB) unique reflections with $F^2 > 2\sigma(F^2)$. Relevant crystallographic data are given in Table 1.

(12) Uekama, K.; Horikawa, T.; Yamanaka, M.; Hirayama, F. *J. Pharm. Pharmacol*. **1994**, *46*, 714−717.
(13) Hirayama, F.; Yamanaka, M.; Horikawa, T.; Uekama, K. *Chem. Pharm. Bull*. **1995**, *43* (1), 130−136.
(14) Hirayama, F.; Horikawa, T.; Yamanaka, M.; Uekama, K. *Pharm. Sci*. **1995**, *1*, 517−520.
(15) Caira, M. R.; Griffith, V. J.; Nassimbeni, L. R.; van Outshoorn, B. *J. Chem. Soc., Perkin Trans. 2* **1994**, 2071−2072.
(16) Steiner, T.; Saenger, W. *Angew. Chem., Int. Ed. Engl*. **1998**, *37*, 3404−3407.
(17) Harata, K.; Hirayama, F.; Arima, H.; Uekama, K.; Miyaji, T. *J. Chem. Soc., Perkin Trans 2* **1992**, 1159−1167.
(18) Harata, K. *J. Chem. Soc., Chem. Commun*. **1988**, 928−929.
(19) Sensse, K.; Cramer, F. *Chem. Ber*. **1969**, *102*, 509−521.
(20) Takeo, K.; Kuge, T. *Starch* **1972**, *24*, 281−284.

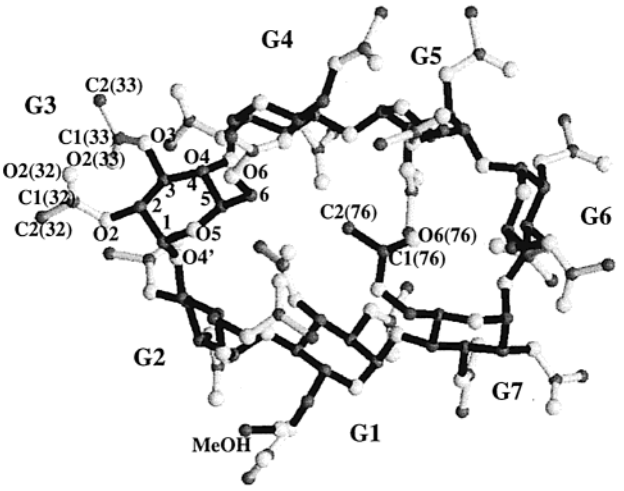

Figure 1. Structure and numbering scheme of TA. C2(76) denotes atom C2 of the acyl chain bonded to O6 of glucose G7. The β-CD backbone and the acyl chain 76 which is included in the macrocycle are drawn in black for clarity, all other acyl chains in white. Oxygen atoms are shown as white spheres and carbon atoms are in gray (β-CD) or black (acyl chain). A $^{O}S_2$-twist glucose (G5) is observed only in TA and TP, whereas the macrocycle in TB is less elliptically distorted and all glucoses are in the $^4C_1$-chair conformation (see also Figure 5a−c, where the orientation of TA, TB, and TP is identical to that shown here for TA. Figure drawn with MOLSCRIPT.[43]

(2) Structure Determination and Refinement. All attempts to determine the crystal structures by conventional direct methods failed, which is not surprising since the success rate of these methods falls off rapidly above 150 non-hydrogen atoms per asymmetric unit (the present crystal structures contain 142 (TA), 161 (TP), and 182 (TB) C and O atoms). The structure of TA was finally obtained by a novel "ab initio" real/reciprocal space recycling procedure[21] implemented in SHELXD inspired by, but different in detail from, the "Shake and Bake" method.[22,23] In structure refinement using F^2 with full matrix least squares (SHELXL97),[24] all non-hydrogen atoms were treated anisotropically and hydrogen atoms were placed at idealized positions (C−H = 1.07 Å) in a riding model; the TA atoms and the cocrystallized MeOH molecule are in well-defined positions. The refinement converged at a final $R = 0.033$ for 6231 reflections with $F_0 > 4\sigma$ (0.044 for all 7339 reflections).

The numbering scheme of the molecules TA, TP, and TB is shown in Figure 1. The atoms of the acyl chains are distinguished from the CD atoms by two numbers in parentheses, the first denoting the number of the glucose unit and the second the number of the glucose atom to which the acyl group is attached.

Since the crystals of TP were nearly isomorphous to TA, the atomic coordinates of TA were used as an initial model to determine the crystal structure using the program SHELXD. The refinement procedure was the same as for TA and converged at a final $R = 0.034$ for 10 051 reflections with $F_0 > 4\sigma$ (0.047 for all 12 346 reflections). All TP atoms are fully occupied except for C3(66) (atom C(3) of the acyl chain bonded to O(6) of glucose 6).

The set of TP atomic coordinates was used as an initial model to obtain the structure of TB using molecular replacement methods (SHELXD). Refinement was similar to that described for TA and converged at a final $R = 0.055$ for 10 508 reflections with $F_0 > 4\sigma$ (0.069 for all 12 367 reflections). Large temperature factors and additional peaks in the difference Fourier maps indicated 2-fold disorder of six C(4)H_3 methyl groups: C4(13) (atom C4 of the acyl chain, bonded to O(3) of glucose 1), C4(26), C4(42), C4(43), C4(62), and C4(73), and one oxygen atom O3(23) were 2-fold disordered with occupation factors of 0.65 and 0.35, the two alternate positions having been refined with the sum of occupation restrained to 1.0. Occupation factors of C4(56) and C4(63) were assessed by inspection of electron densities (Program XtalView[25]) as 0.5, 0.5, and C3(16), as 0.8, 0.2, respectively.

Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications no. CCDC-163108 (TA), CCDC-163109 (TP), and CCDC-163110 (TB). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax, +44 1223 336033; e-mail, deposit@ccdc.cam.ac.uk).

(3) Spectroscopic Investigation. For circular dichroism (cd) spectroscopic investigations, we prepared 2.5 mM solutions of TA, TP, and TB in ethanol and added a constant amount of a stock solution (to a final concentration of 10 mM) containing the desired guest molecules (molsidomine, *m*-iodophenole, 2,7-dihydroxynaphthalene, and hydroquinone) also dissolved in ethanol. The solutions were mixed, kept overnight to reach equilibrium, and investigated by cd spectroscopy. The cd spectra were recorded in the wavelength range 200−350 nm using a Jasco cd spectrometer J600 with cuvettes of 0.1 cm path length.

Results

Molecular Structure and Conformational Analysis. (a) Conformation of the Macrocycle. Geometrical parameters describing the overall shapes of the macrocycles are shown in Tables 2 and 3. The macrocycles of TA, TP, and TB are elliptically distorted (Figures 1, 2a−c) with major and minor diameters of ellipses (measured between O4(*n*) atoms) given in Table 2. The elliptical shape is also reflected in the large differences in O4(*n*)···O4(*n*−1) distances; i.e., the distances O4(1)···O4(7) and O4(5)···O4(4) (glucoses G1 and G5) are stretched for TA, TP, and TB in the range 4.49−4.71 Å (see Table 2), whereas O4(3)···O4(2) (glucoses G3) are short, 3.88 Å in TA and 3.98 Å in TP due to kinks between glucoses G3 and G2 (see large tilt angles of 50.9 and 48.2° for G3 in TA and TP, respectively, in Table 2, while the tilt angle for TB is different, −4.9°).

The O4(*n*)−O4(*n*+1)−O4(*n*+2) angles cover wide ranges from 99.2−150.7° for TA, 104.8−145.4° for TP, and 106.6−145.8° for TB, again reflecting the elliptical distortion and giving rise to boat-shaped structures of the macrocycle. As Figure 2 illustrates, the macrocycles are more distorted in TA and TP than in TB.

The absence of O2···O3′ hydrogen bonding gives rise to wide O2(*n*)−O3(*n*−1) distances in TA, TP, and TB which greatly exceed the average value (2.88 Å) for β-CD·11H_2O.[26,27] It explains why the torsion angles ϕ and ψ describing the orientation of the glucoses with respect to their glycosidic link vary to such a large extent (Table 3) and why some of the glucose residues are severely tilted to the O4-plane, defined as the least-squares plane containing all seven O4 atoms; see Table 2. In TA and TP the tilt angles are comparable, but the pattern is different in TB. As the superposition of the three molecules in Figure 3 shows, the glucose units and acyl chains in TA and TP have similar orientations, whereas in TB some of the glucose units and acyl chains are oriented differently. After superimposition of the macrocycles (omitting the acyl chains), the resulting root mean square deviations reflect these features: the values

(21) Sheldrick, G. M. January 1997, Collaborative Crystallographic Project 4 (CCP4) Meeting, University York/UK.

(22) Miller, R.; DeTitta, G. T.; Jones, R.; Langs, D. A.; Weeks, C. M.; Hauptmann, H. A. *Science* **1993**, *259*, 1430−1433.

(23) Miller, R.; Gallo, S. M.; Khalak, H. G.; Weeks, C. M. *J. Appl. Crystallogr*. **1994**, *27*, 613−621.

(24) Sheldrick, G. M.; Schneider, T. R. SHELXL: High-Resolution Refinement. In *Methods in Enzymology*, Vol. 277; Carter, C. W., Jr., Sweet, R. M., Eds.; Academic Press: San Diego, 1997; pp 319−343.

(25) McRee, E. D. *Practical Protein Crystallography*; Acedemic Press: San Diego, 1993.

(26) Betzel, C.; Saenger, W.; Hingerty, B. E.; Brown, G. M. *J. Am. Chem. Soc*. **1984**, *106*, 7545−7557.

(27) Steiner, T.; Mason, S. A.; Saenger, W. *J. Am. Chem. Soc*. **1991**, *113*, 5676−5687.

Table 2. Geometrical Data for TA, TP, and TB

	diam O4(3)−O4(6) × O4(4)−O4(7) (Å)	O4(*n*)···O4(*n*−1) (Å)	glycosidic angles C4(*n*)−O4(*n*)−C1(*n*+1) (deg)	O4(*n*)···O4(*n*+1)···O4(*n*+2) (deg)	O2(*n*)···O3(*n*−1) (Å)	tilt angles[a] (deg)
TA	17.4 × 10.4					
G1		4.61	117.0	99.2	3.26	−34.8
G2		4.36	121.2	130.5	3.85	14.7
G3		3.88	117.2	150.7	3.96	50.9
G4		4.40	116.8	113.7	4.28	−16.8
G5		4.82	119.0	104.5	4.56	11.6
G6		4.13	120.6	149.4	3.73	−25.6
G7		4.01	118.3	139.6	4.17	42.0
TP	16.4 × 11.2					
G1		4.49	116.8	104.8	3.43	38.0
G2		4.32	120.1	130.6	3.69	18.2
G3		3.98	116.0	142.7	3.89	48.2
G4		4.43	118.8	118.4	4.17	11.9
G5		4.71	116.9	107.5	3.22	14.5
G6		4.33	120.3	145.4	3.67	25.9
G7		4.22	117.4	138.5	4.10	37.1
TB	16.2 × 13.2					
G1		4.62	116.3	122.4	3.56	−29.8
G2		4.27	116.4	117.0	3.77	32.5
G3		4.37	118.4	145.8	3.62	4.9
G4		4.22	116.9	129.2	3.86	34.3
G5		4.60	118.1	106.6	3.24	−42.1
G6		4.16	121.0	143.5	3.76	−15.7
G7		4.25	114.6	130.5	4.16	46.8
β-CD*b*	7.8 × 7.8	4.385	118.7	128.3	2.884	

[a] Tilt angle, which is defined as the angle made by the O4 plane and the plane through C1(*n*), C4(*n*), O4(*n*), and O4(*n*+1). A positive value indicates that the residue is rotated with its O(6) side toward the inside of the molecule. [b] β-CD·11H2O.[18,26]

Table 3

	glucose puckering params[a]			torsion angles[c]		exocyclic torsion angles, χ		
	QT (Å)	θ_2[b] (deg)	ϕ_2[b] (deg)	ϕ (deg)	Ψ (deg)	χ_5(O5(*n*)−C5(*n*)−C6(*n*)−O6(*n*)) (deg)	χ_2(C1(n)−C2(n)−O2(n)−C1(chain)) (deg)	χ_3(C2(n)−C3(n)−O3(n)−C1(chain)) (deg)
TA								
G1	0.592	9.40	−146.6	128	151	−65.5	82.9	−142.0
G2	0.571	9.63	−13.1	96	166	58.4	68.2	−122.1
G3	0.557	17.79	66.0	88	92	−56.4	141.6	−87.6
G4	0.572	1.89	−10.4	99	130	−79.1	81.3	−136.5
G5	0.746	90.22	−35.4	107	125	69.9	149.6	−164.9
G6	0.574	7.08	102.5	96	163	−49.3	128.9	−122.7
G7	0.595	4.43	39.6	79	78	149.4	123.7	−103.6
TP								
G1	0.592	4.54	−142.5	132	156	−63.8	100.2	−138.1
G2	0.544	9.15	16.5	100	157	59.4	79.4	−110.3
G3	0.540	19.66	72.5	87	98	−63.1	140.8	−85.5
G4	0.569	1.22	−4.2	98	136	−78.4	78.7	−130.8
G5	0.734	87.17	−32.7	108	117	66.4	147.2	−160.4
G6	0.563	3.53	91.6	97	157	−43.9	123.2	−119.4
G7	0.582	6.49	42.2	81	79	155.1	133.6	−109.7
TB								
G1	0.572	9.40	−82.2	116	159	−64.3	75.2	−131.0
G2	0.539	10.94	46.9	87	116	61.7	78.3	−95.5
G3	0.564	4.51	77.7	108	158	−71.0	83.9	−128.4
G4	0.581	6.91	77.2	87	83	−58.9	136.0	−91.4
G5	0.567	7.95	−143.5	124	148	−68.9	90.5	−128.2
G6	0.572	8.96	49.5	99	172	−81.6	75.9	−132.4
G7	0.579	5.97	10.5	74	92	146.3	134.1	−94.2
sucr[d]	0.556			109.8	127.6			

[a] Cremer and Pople parameters.[29] [b] Spherical polar angles. [c] According to IUPAC rules:[28] ϕ = O5(*n*+1)−C1(*n*+1)−O4(*n*)−C4(*n*) and Ψ = C1(*n*+1)−O4(*n*)−C4(*n*)−C3(*n*). [d] Taken from ref 29.

are 0.31 Å for TA/TP, 1.4 Å for TA/TB (omitting G5), and 1.32 Å for TP/TB (also omitting G5).

(b) Conformation of the 2,3,6-Tri-*O*-acylglucose Residues. Torsion-angle index[28] and Pople and Cremer ring puckering parameters[29,30] allow the evaluation of differences in pyranose conformation. The glucose puckering parameters (QT), given in Table 3, reflect the relatively unstrained $^4C_1$ conformations (average: 0.577 Å (TA), 0.565 Å (TP), and 0.568 Å (TB)) of

(28) IUPAC. *Eur. J. Biochem.* **1983**, *131*, 5−8.

(29) Cremer, C.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354−1358.

(30) Nardelli, M. *Comput. Chem.* **1983**, *7*, 95.

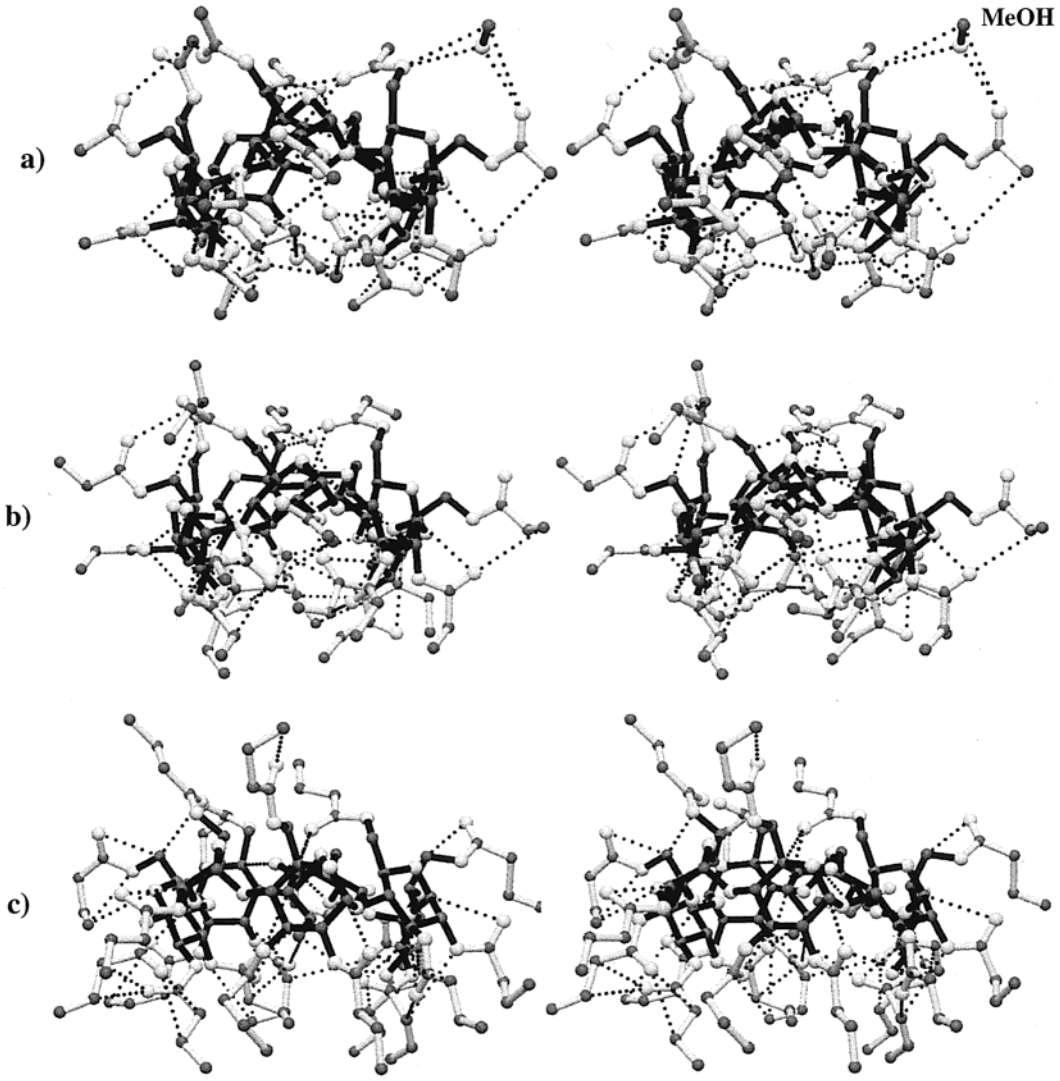

Figure 2. Side views in stereo, glucoses G3 in front of TA (a), TP (b), and TB (c) emphasizing the boat-shaped structure of the molecules. The acyl chains close the cavities of TA and TP from both sides such as a lid but the O2, O3 side of TB is more open. Dotted lines show the network of intramolecular C−H···O hydrogen bonds which stabilize the position of the acyl chains. Drawn with MOLSCRIPT.[43]

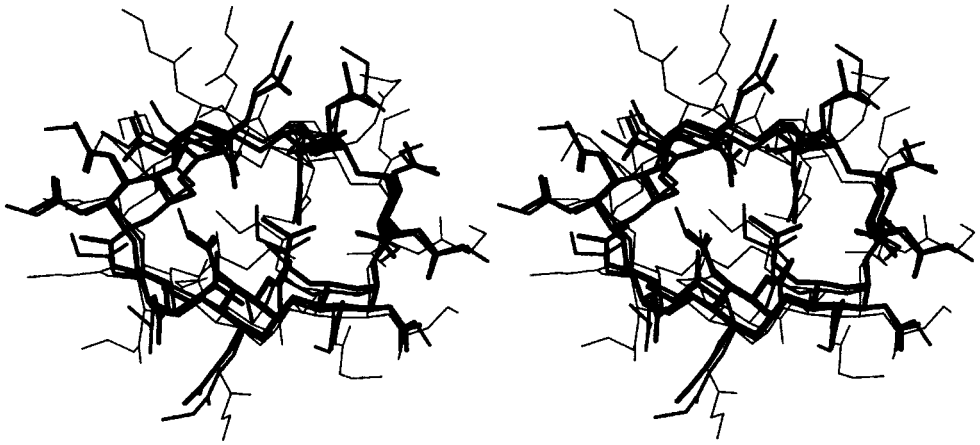

Figure 3. Stereo drawing of the superimposition of TA (thick line), TP (medium line), and TB (thin line). O6 side of β-CD toward the viewer. Drawn with MOLSCRIPT.[43]

all glucose residues except for the G5 glucoses in TA and TP, 0.746 and 0.734 Å, respectively. The latter glucoses are in the $^{o}S_2$ skew-boat (twist) conformation with ϕ_2 values of 90.22 and 87.17° for TA and TP, respectively, contrasting the normally found $^4C_1$ chair form (Figure 4).

The torsion angles χ_5(O5−C5−C6−O6) describing rotation about the C5−C6 bonds are in the preferred (−)gauche conformation for glucoses 1, 3, 4, and 6 in TA and TP, and 1 and 3−6 in TB, and in (+)gauche conformation (O6 is pointing away from the center of the cavity) at glucoses 2 and 5 for TA

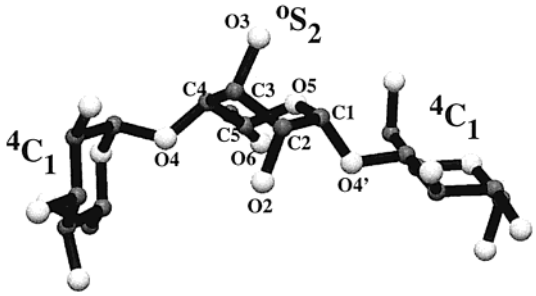

Figure 4. $^{O}S_2$-skew-boat conformation of the G5 unit (center) in TA and TP and the $^4C_1$-chair conformation (left and right). Carbon atoms in dark gray and oxygen atoms in white. In the $^{O}S_2$ conformation the C2−O2, C3−O3, and C4−O4 bonds are pseudoaxial and the C2−O2 and C4−O4 bonds are trans to the C3−O3 bond, contrasting the $^4C_1$-chair form. Drawn with MOLSCRIPT.[43]

and TP, and at glucose 2 for TB. The χ_5 torsion angle at glucose 7 is in trans-conformation with $\chi_5 = 149$, 155, and 146° for TA, TP, and TB, respectively; this conformation has never been observed in a CD crystal structure before. Further torsion angles, χ_2(C1(*n*)−C2(*n*)−O2(*n*)−C1(chain)) are (+)gauche to trans and χ_3(C2(*n*)−C3(*n*)−O3(*n*)−C1(chain)) are (−)gauche to trans (Table 3).

(c) Network of C−H···O Hydrogen Bonds. In the peracylated β-CDs, the macrocyclic structure is stabilized by inter- and intramolecular C−H···O hydrogen bonds between adjacent acylglucose units (Figure 5); they are a common feature found in carbohydrate crystal structures with a high density of acceptor O atoms.[31] The commonly used hydrogen bond parameters $d_{C\cdots O}$, $d_{H\cdots O}$, and $\alpha_{CH\cdots O}$ served to define cutoffs with $d_{H\cdots O}$ up to 3.0 Å and $\alpha_{CH\cdots O} \geq 100°$. Hydrogens bonds involving disordered C-atoms will not be discussed.

Of the 21 potential ester (carbonyl) O acceptor atoms in each of the three crystal structures, 19 are engaged in C−H···O hydrogen bonds, and in some cases a carbonyl oxygen atom accepts two C−H donors. In total there are 55 (TA), 51 (TP), and 52 (TB) C−H···O hydrogen bonds (involving glucose O2, O3, O4, O6, and carbonyl O), indicating that the length of the acyl chain does not affect the number of C−H···O contacts.

Although the number of potential C−H donors of the acyl chains increases from TA (63), to TP (105), and TB (147) due to the growing number of CH_2 groups in the chains, the number of intramolecular C−H···O bonds in which these H-atoms are involved are as follows: 20 (TA), 14 (TP), and 19 (TB). The reason the number of C−H···O hydrogen bonds does not increase with the acyl chain lengths is that the "ends" of the chains become more distant with increasing chain length, so that intramolecular hydrogen bonds cannot be formed.

In TA and TP, acyl chain 76 is located "over" the CD cavity like a lid, but it penetrates into the cavity in TB. It is stabilized in this position by five intramolecular C−H···O hydrogen bonds in TA and TP and by six in TB (Figure 5a−c), and the ester carbonyl oxygens accept C−H···O hydrogen bonds, C5(7)−H···O6(76) and C5(6)−H···O6(76). These interactions are only possible because the torsion angle χ_5(O5(7)−C5(7)−C6(7)−O6(7)) is in the unusual trans form, so that the acyl chain at G7 is in an orientation suitable to be located "over" (TA, TP) or insert into (TB) the β-CD cavity.

All acyl chains are integrated into a network of intra- and/or intermolecular C−H···O hydrogen bonds. In addition there are C−H···O contacts between adjacent glucose units, which are characteristic for carbohydrates.[32] The interactions C5(*n*)−H···O4(*n*−1) and C6(*n*)−H6(*n*)···O5(*n*+1) are found between all glucose units, with H···O distances in the ranges 2.31−2.93 and 2.22−2.97 Å, respectively.

The cocrystallized MeOH in TA forms two O−H···O hydrogen bonds (MeO···O6(2), 2.76 Å; MeO···O6(16), 2.82 Å) and two C−H···O hydrogen bonds (Me-C···O6(2), 3.47 Å; Me-C···O6(16), 3.51 Å) with the acyl chains (Figure 2a).

(d) Crystal Packing. In the crystal structures of TA and TP (Figure 6a,b) the molecules are stacked head-to-tail along the *a*-axis to form infinite columns. In contrast to the common channel structures formed by CD inclusion complexes,[40−42] adjacent columns in TA and TP are antiparallel. The packing of TA and TP molecules in the stacks is comparable, as shown by the similar lengths of the *a*-axes, 11.567(8) Å in TA and 12.562(8) Å in TP. In the crystal structure of TA, one MeOH per TA molecule is located in interstices between the stacks (Figure 6a). In TP, no solvent molecule is found which we associate with the longer acyl chains filling the interstices.

In TB, the molecules are also stacked in head-to-tail arrangement along *a*, but adjacent molecules are laterally displaced leading to a zipperlike organization (Figure 6c). Along *b*, the zippers are in antiparallel orientation. Adjacent molecules are closely packed in "dimer" form, and the interstices between the dimers are filled by the acyl side chains.

Complexing Ability. For the characterization of the complexing behavior of CDs, cd-spectroscopy is a suitable method.[19,20] Achiral guest molecules containing a chromophore show an induced Cotton effect upon inclusion in a chiral CD, which can be determined at the absorption maximum of the chromophore. The chosen guest molecules (with absorption maxima in parentheses) were molsidomine (231.6 and 319 nm), *m*-iodophenole (235 nm), 2,7-dihydroxynaphthalene (235 nm), and hydroquinone (227 and 295 nm).

Solutions of the CDs and the guest molecules were mixed as described in Experimental Section, and the cd spectra of these mixtures were compared with the spectra of solutions of the corresponding individual compounds. No formation of inclusion complexes could be observed; i.e., the spectra of the mixtures were in all cases linear combinations of the spectra of the individual compounds. Thus, the acyl side chains severely interfere with the ability of peracylated β-CDs to form inclusion complexes.

Discussion

Tilting of Pyranose Units. In contrast to unsubstituted CDs, the structures described here are not stabilized via O2(*n*)···O3(*n*−1) hydrogen bonds as all hydroxyl groups have been

(31) Steiner, T.; Saenger, W. *J. Am. Chem. Soc*. **1992**, *114*, 10146−10154.
(32) Etter, M. C.; MacDonald, J. C.; Bernstein, J. *Acta Crystallogr*. **1990**, *B46*, 256−262.
(33) Harata, K. *Chem. Lett.* **1998**, 589−590.
(34) Dowd, M. K.; French, A. D.; Reilly, P. J. *Carbohydr. Res.* **1994**, *264*, 1−19.
(35) Bentley, R. *Annu. Rev. Biochem.* **1972**, *41*, 953−957.
(36) Harata, K.; Otagiri, K.; Hirayama, F. *Bull. Chem. Soc. Jpn*. **1983**, 56, 1732−1736.
(37) Di Blasio, B.; Galdiero, S.; Saviano, M.; de Simone, G.; Benedetti, E.; Pedone, C.; Gibbons, G. A.; Deschenaux, R.; Rizzarelli, E.; Vecchio, G. *Supramol* **1996**, *7*, 47−50.
(38) Di Blasio, B.; Pavone, V.; Nastri, F.; Isernia, C.; Saviano, M.; Pedone, C.; Cucinotta, V.; Impellizzeri, G.; Rizzarelli, E.; Vecchio, G. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *98*, 7218−7221.
(39) Wulff, G.; Steinert, A.; Höller, O. *Carbohydr. Res*. **1998**, *307*, 19−31.
(40) Harding, M. M.; Maclennan, J. M.; Paton, R. M. *Nature* **1978**, *274*, 621−623.
(41) Jogun, K. H.; Stezowski, J. J. *Nature* **1979**, *278*, 667−668.
(42) Betzel, C.; Hingerty, M.; Noltemeyer, M.; Weber, G.; Saenger, W. *J. Inclusion Phenom.* **1983**, *1*, 181−191.
(43) Kraulis, P. J. *J. Appl. Crystallogr*. **1991**, *24*, 946−950.
(44) *INSIGHTII*, 97.0; Molecular Simulations: San Diego, 1997.

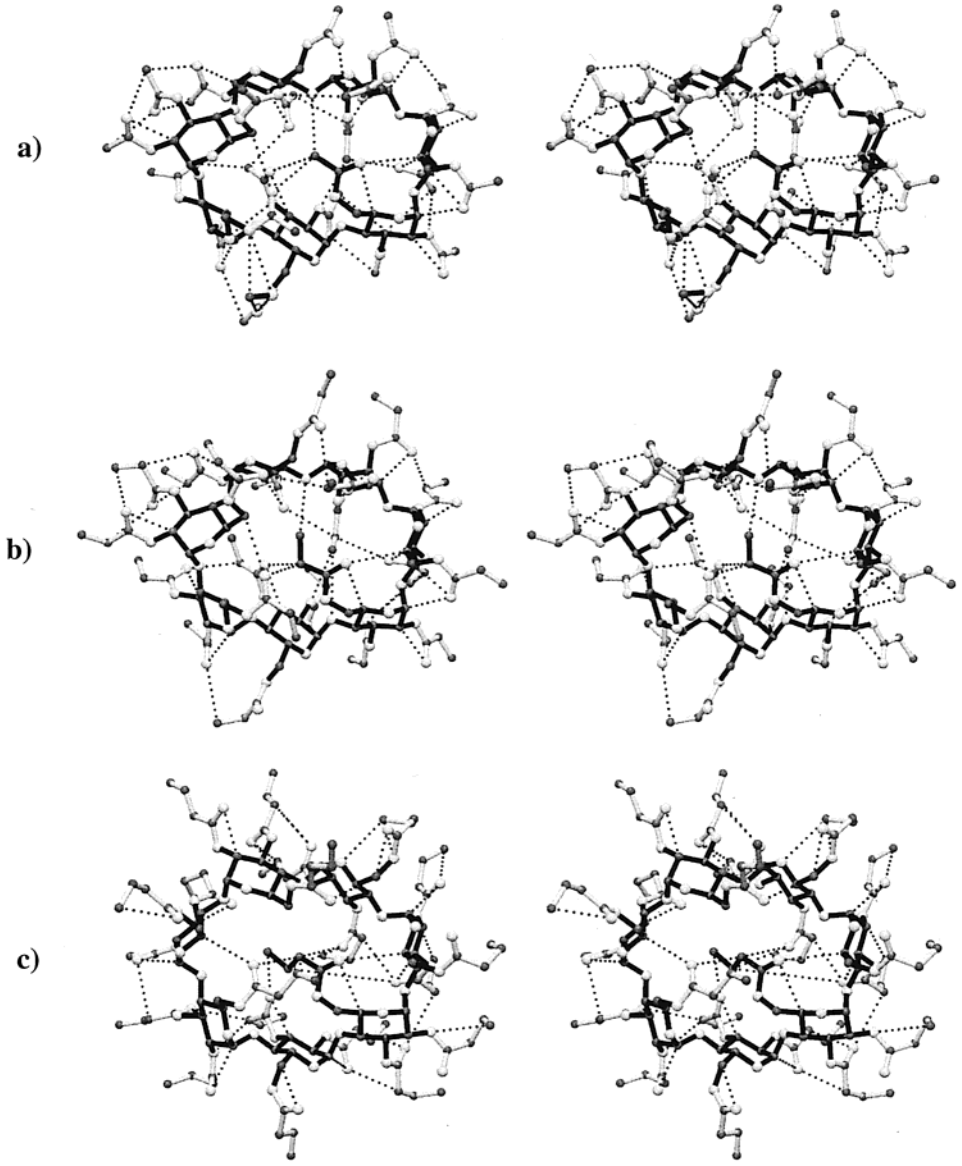

Figure 5. Stereo plot of top views of TA (a), TP (b), and TB (c). The dotted lines show the network of C−H···O hydrogen bonds which stabilize the macrocyclic structures. Drawn with MOLSRIPT.[43]

chemically modified by acyl chains of increasing lengths in the series TA, TP, and TB. This is accompanied by a pronounced distortion of the macromolecular conformation due to steric hindrance between acyl chains, mainly on the O2, O3 side of the molecule. To relieve this steric hindrance, the O2···O3′ distances increase, with averages in TA, TP, and TB of 3.97, 3.74, and 3.71 Å, respectively, significantly larger than the average value found in unsubstituted β-CDs (2.88 Å).[26] As evidenced by the glycosidic torsion angles and tilt angles (Table 2), the increase of the O2···O3′ distances is associated with an inclination of individual glucose residues; i.e., the steric hindrance caused by the acyl chains has a direct impact on the macromolecular structures.

A comparable effect of trisubstitution is also found in the smaller α-CD analogue, hexakis(2,3,6-tri-*O*-acetyl)-α-CD·H_2O featuring an elliptically distorted cavity.[33]

$^{o}S_2$ Conformation. The structures presented herein demonstrate that peracylation not only changes the overall shape of β-CD macrocycles but may modify the conformation of individual pyranose rings. One possibility for reducing steric hindrance due to the presence of acyl chains is the conformational change of a glucose unit from the $^4C_1$-chair conformation to the $^{o}S_2$-skew-boat conformation (a high-energy intermediate state between the $^4C_1$ and $^1C_4$ conformations[34,35]), as seen in the G5 units of TA and TP. Their C1, C2, C4, and C5 atoms form a twisted plane so that the C3 and O5 atoms are exoplanar. With the change from $^4C_1$ to $^{o}S_2$, the pseudoequatorial bonds C2−O2, C3−O3, and C4−O4 change into a pseudoaxial orientation, while the C1−O4′ bond changes from pseudoaxial to pseudoequatorial. As a result, the C3−O3 bond is trans to the C2−O2 and C4−O4 bonds and the attached acyl chains point in different directions, thereby reducing steric hindrance (Figure 4).

In crystal structures of substituted CDs, glucoses with conformations other than $^4C_1$ have been observed before in uncomplexed permethylated β-CD (TRIMEB), where one of the trimethylglucose units is converted from the $^4C_1$- to the $^1C_4$-chair form with pseudoaxial C2−O2, C3−O3, and C5−C6 bonds.[15] If complexed with *m*-iodophenol, one trimethylglucose unit of TRIMEB adopts also the $^{o}S_2$ skew-boat conformation.[17,18] This contrasts TRIMEB in the *p*-iodophenol complex, in which all trimethylglucose units are in the $^4C_1$ chair conformation.[36]

Orientations of Acyl Chains: Not Random and Associated with $^{O}S_2$ Glucose Conformation. As described under Results,

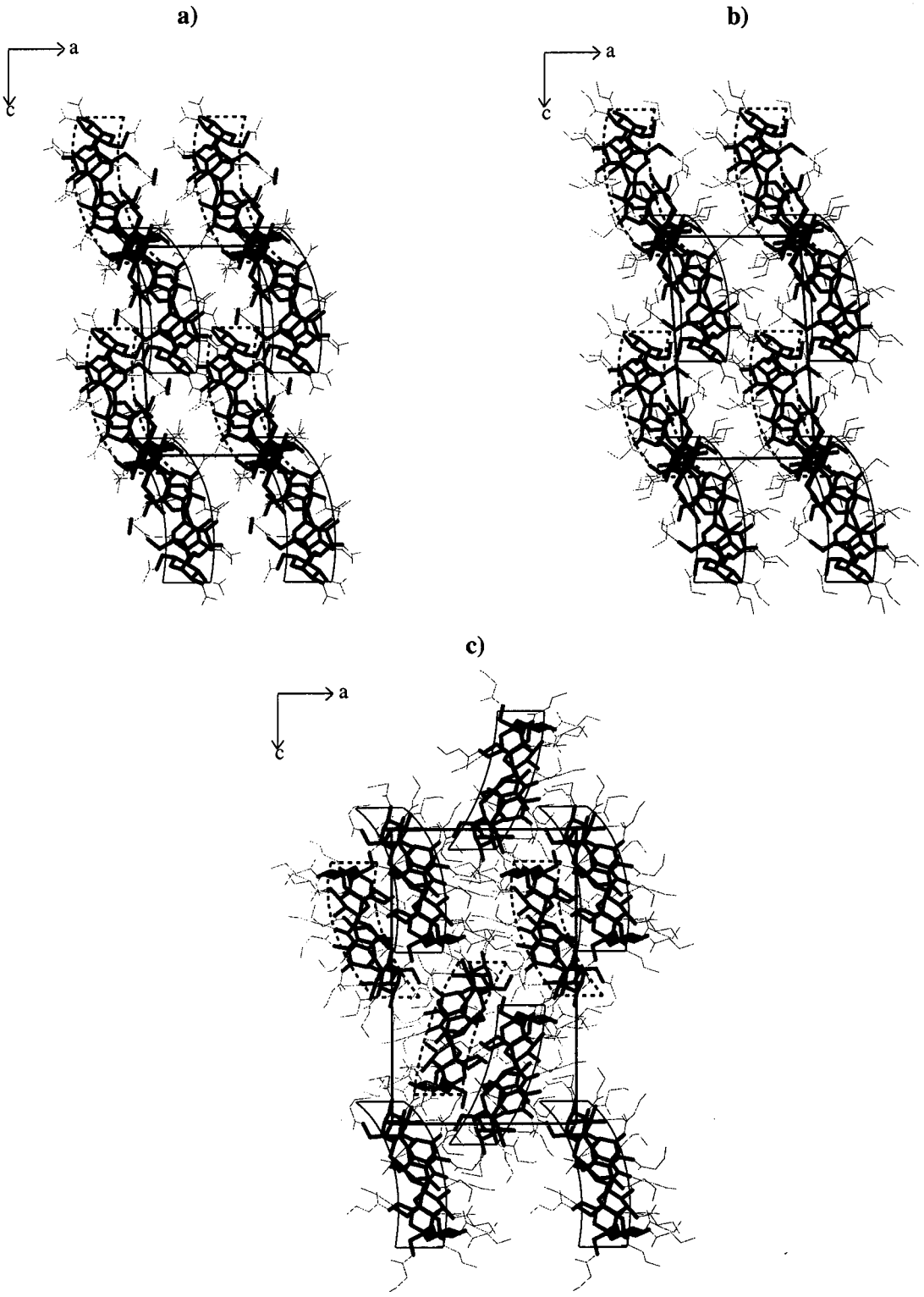

Figure 6. Crystal packing of TA (a), TP (b), and TB (c), viewed along the b axes. Thick lines denote the β-CD backbone and thin lines the acyl chains. Drawn with INSIGHTII.[44]

the conformations of the β-CD macrocycles are similar for TA and TP but different for TB. This we associate with the orientations of the acyl chains, which are also similar for TA and TP but different for TB.

The main determinant for the molecular structures of TA, TP, and TB appears to be the length and the orientation of acyl chains bound to O6(7). In TA and TP, they close the molecular cavities from the O6 side but do not enter the cavity, probably because they are too short. They form comparable C−H···O hydrogen bonds with acyl CH_3 (TA) and CH_2–CH_3 (TP), donating to O4(4) across the cavity and to O2(13) and O2(26), while O2(76) acts as acceptor for C5(6)−H and C5(7)−H, Figures 2 and 5.

By contrast, the longer butyryl chain at O6(7) of TB penetrates deeply into the cavity, with O2(76) accepting C−H···O hydrogen bonds similar to TA and TP, but the CH_2−CH_2−CH_3 chain interacts only with acyl carbonyl oxygen atoms O2(13) and O2(53).

The orientations of the acyl chains at the O2, O3 side are again comparable in TA and TP but different in TB; see Figures 2 and 5. Since there are twice as many of these acyl chains compared to the O6 side, they are engaged in more intramolecular contacts. In TA and TP, acyl chains interact across the cavity, thereby closing it (TA, C2(13)−H···O2(43)/O2(23) and C2(52)−H···O2(1)/O2(63); TP, C2(13)−H···O2(23) and C3(52)−H···O2(1)/O4(7)). In TB, there are only a few contacts across the cavity, and they are not direct but mediated by the enclosed butyryl chain. This and the enclosure of the butyryl chain as guest might be the reason the β-CD macrocycle in TB is less elliptically deformed than in TA, TP.

In this spirit, the unusual $^{O}S_2$ conformation of G5 in TA, TP is probably associated with the "empty" cavity. Its volume is reduced by C−H···O hydrogen bonds across the cavity at the O6 and O2, O3 sides, which involve mainly G5 and facilitate conformational change to $^{O}S_2$. In TB, however, the cavity is filled by the butyryl chain at O6(7), and there are no or few C−H···O bonds across the cavity, which is in a more open form than in TA, TP, and all glucoses are in the $^4C_1$ conformation.

Formation of Inclusion Complexes. The self-inclusion of TA, TP, and TB will possibly prevent the inclusion of any other guest molecule into the central cavity. This is similar to acetylated amyloses whose ability to form inclusion complexes decreases with increasing degree of substitution, and the specificity to form inclusion complexes depends on the substituents: acetylated amyloses have a remarkably higher specificity than amyloses modified by hydroxypropyl, hydroxyethyl, and 1,2-dihydroxypropy groups.[39] Another reason for the poor inclusion formation of TA, TP, and TB might be their low solubility in water. In all the studies mentioned here, the acylated β-CDs were dissolved in MeOH:H_2O (8:2). Since MeOH is known to form inclusion complexes with CD, it will compete with other potential guests for the cavities in TA, TP, and TB. The cavities of these molecules, however, are not filled by H_2O or MeOH in the here described crystal structures

Conclusions

Compared to unsubstituted CDs, the physical and structural properties of the presented peracylated CD homologues are markedly changed. They show atypical behavior, in that they are insoluble in water and—as shown in this work—are unable to form the classical inclusion complexes. In crystals or precipitates of the peracylated CDs, guest molecules can only be accommodated in the matrix between the acyl chains and not in the central cavity. If complexed with peracylated CD by the kneading method, the water-soluble drug molsidomine adopts the insolubility in water of the substituted CDs, so that the rate of drug release is retarded with increasing order of the hydrophobicity of the host molecules. Among the peracylated β-CDs tested, TB maintained sufficient plasma drug levels for a long period of time, while other peracylated β-CDs having shorter or longer chains were inappropriate to control the in-vivo release behavior of molsidomine. The prominent retarding effect of TB was ascribed to the mucoadhesive property and hydrophobicity that differs from other peracylated β-CDs.

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