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The crystal structure of the inclusion complex of cyclomaltoheptaose (β-cyclodextrin) with 3,3-dimethylbutylamine

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ABSTRACT

The crystal of the 1:1 complex of 3,3-dimethylbutylamine with cyclomaltoheptaose (βCD, β-cyclodextrin) is monoclinic $C2$ with $a = 19.187(9)$, $b = 24.56(1)$, $c = 15.893(7)$ Å, $\beta = 108.77(4)°$, $V = 7091$ Å^3, and $Z = 4$. Two βCD molecules, held together by intermolecular hydrogen bonds involving HO-3, form dimers, in the cavities of which two 3,3-dimethylbutylamine and two water molecules are accommodated. The guest molecule is completely enclosed in the cavity. The amino group is located at the secondary-hydroxyl-group side, and is hydrogen-bonded to the entrapped water molecules. The dimers form channels along the c axis. The inter-dimer space is filled with 10.7 water molecules that are distributed over 14 sites, and there is a dense network of hydrogen bonds involving the water molecules and the βCD hydroxyl groups.

INTRODUCTION

The cyclomalto-oligosaccharides (cyclodextrins, CDs) show a remarkable ability to form inclusion complexes with various natural and synthetic molecules. The inclusion process is influenced not only by the host–guest hydrophobic interactions but also by the shape, size, and hydrogen-bonding ability of the guest. Depending on the size and molecular or ionic character of the guest, αCD inclusion complexes crystallise in distinct categories[1] and most βCD inclusion complexes crystallise as dimers in four modes[2].

The title inclusion complex is one of several with a guest that can be located readily, and which have been selected in order to study systematically the characteristics of the guest on the geometry of the inclusion complex, the formation of the dimers, and the packing of the βCD macrocycle.

EXPERIMENTAL

Preparation of crystals and X-ray measurements. — To an aqueous solution of βCD was added 3,3-dimethylbutylamine until the host–guest ratio was 1:10. The

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complex, which precipitated at room temperature, was redissolved in water at 75°, a 10-fold excess of 3,3-dimethylbutylamine was added, and the solution was allowed to cool slowly (4 days) to give colorless prisms.

A crystal (0.25 × 0.5 × 0.5 mm) was sealed in a thin-walled glass capillary in order to prevent loss of water. One quadrant of data $3° < 2\theta < 44°$ was collected on a syntex $P2_1$ diffractometer, with Nb-filtered Mo-K_α radiation, with a θ–2θ scan mode at scan rates in the range 1.0–10°/min and a scan width of 1.8° plus the α_1–α_2 divergence. The background was measured at the start and end of each scan for 25% of the scan time of each reflection. The decay of the crystal was monitored by three standard reflections ($16° < 2\theta < 18°$), but no decay was noted. No absorption correction was considered to be necessary ($\mu = 0.79$ cm^{-1}). The intensities were corrected for Lorentz and polarisation effects. Of the 4485 unique reflections, 2891 with $F > 5\sigma(F)$ were used in the refinement of the structure.

Crystal data. — From the final results, the composition inferred was $C_{42}H_{70}O_{35}{\cdot}C_6H_{15}N{\cdot}(H_2O)_{10.7}$. The final lattice parameters, as determined by the centering of 15 reflections ($16 < 2\theta < 20°$), were as follows: monoclinic, space group $C2$, a = 19.187(9), b = 24.56(1), c = 15.893(7) Å, β = 108.77(4)°, V = 7091 Å^3, Z = 4, and D_{calc} = 1.34 g/cm^3.

Determination of the structure and refinement. — The structure is isomorphous with that of the inclusion complex of βCD and benzophenone or biphenyl[3]. Therefore, the initial co-ordinates used for the βCD molecule were those of the βCD–benzophenone complex. Fourier techniques were used extensively in order to locate the water molecules and the guest atoms within the host lattice. The refinement was carried out by full-matrix least-squares (SHELX 76[4]) techniques minimising $\Sigma w\,(F_o - F_c)^2$ with unit weights for all reflections. It was crucial that the latter started after all non-hydrogen atoms had been located. At the beginning of the refinement, the occupancy factor of the guest was allowed to vary but, since its value was very close to unity, it was set at one and was not refined subsequently. The occupancy factors of the water molecules were refined. For three water molecules, they converged to a value > 1 and they were kept constant at one. Towards the end of the refinement, calculated hydrogen atoms (H-atoms) linked to carbon atoms were used; 13 hydroxyl H-atoms were also located, but their positions were not refined. Isotropic thermal parameters were used for all atoms throughout the refinement except in the last least-squares cycles, where all the oxygen atoms of βCD and some water oxygen atoms were assigned anisotropic temperature factors. The refinement converged to R = 0.0786 and R_w = 0.0878 for observed reflections, and R = 0.1222 and R_w = 0.1163 for all reflections. Maximum and minimum values in the final difference-electron-density map were 0.36 and −0.26 e Å^{-3}, respectively. The number of refined parameters was 556. The final atomic co-ordinates are listed in Table I*.

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* Tables of positional and isotropic thermal parameters for hydrogen atoms and thermal parameters for non-hydrogen atoms are deposited with, and can be obtained from, Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to BBA/DD 474/*Carbohydr. Res.*, 220 (1991) 11–21.

DISCUSSION

The numbering scheme adopted for the complex is given in Fig. 1. C-mn or O-mn denotes the mth atom within the nth glucosidic residue (G-n). Two complex molecules, related by the two-fold crystal axis, form a head-to-head dimer by means of O-3n···O-3′(8-n) hydrogen bonds. The guest molecule is accommodated in the cavity of the βCD dimer.

Bond lengths, bond angles, and torsion angles of the βCD molecule are given in Tables II and III. The pyranose rings have the $^4C_1$ conformation. Except for units G-4/6, the C-6n–O-6n bonds are *gauche* to the C-4n–C-5n and C-5n–O-5n bonds and point out of the cavity. In units G-4/6, the primary hydroxyl groups are disordered and have major (A, pointing outwards) and minor (B, pointing inwards) orientations (occupation factors 0.28, 0.28, and 0.37, respectively). In the inward orientation, the C-6n–O-6n bond is *anti* to the C-4n–C-5n bond and *gauche* to the C-5n–O-5n bond.

The βCD molecule shows seven-fold symmetry, which is reflected in the O-4n···O-4(n+1) distances and O-4(n−1)···O-4n···O-4(n+1) angles (Fig. 2) as well as the small deviations of the O-4n atoms from the optium plane shown along with some other parameters in Table IV. The tilt and dihedral angles indicate that the primary-hydroxyl-group side of the cavity is a little narrower than the secondary-hydroxyl-group side.

The conformation of the macrocycle is stabilised through strong intramolecular hydrogen bonds. The distances between the O-3 of one glucosidic unit and the O-2 of the next, *i.e.*, O-3n···O-2(n+1) (Fig. 2), averaged 2.80 Å, and the angles C-3n–

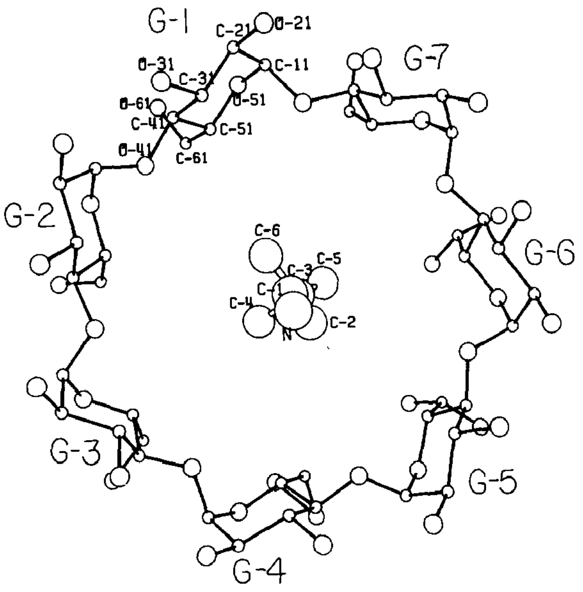

Fig. 1. The structure and numbering scheme of the complex of βCD with 3,3-dimethylbutylamine.

TABLE I

Positional (× 10^4) and thermal parameters ($U^2 = B/8\pi^2$, × 10^3) of the non-hydrogen atoms with e.s.d.'s in parentheses, and the occupation factors (K) for the water molecules

Atom	x	y	z	U^a	*K*
C-11	−585(9)	2700(8)	2470(10)	57	
C-21	−952(9)	2762(8)	3170(10)	58	
O-21	−451(6)	2873(5)	3998(7)	61 *	
C-31	−1384(8)	2259(7)	3170(10)	48	
O-31	−1818(6)	2309(5)	3774(7)	59 *	
C-41	−1934(9)	2145(7)	2230(10)	51	
O-41	−2308(6)	1638(4)	2249(7)	51*	
C-51	−1492(9)	2110(8)	1590(10)	63	
O-51	−1073(6)	2594(5)	1610(8)	62 *	
C-61	−1990(10)	2040(10)	620(20)	102	
O-61	−2448(9)	2448(8)	340(10)	128 *	
C-12	−3080(10)	1631(8)	1900(10)	57	
C-22	−3380(10)	1419(7)	2590(10)	56	
O-22	−3144(6)	1737(4)	3383(8)	64*	
C-32	−3134(9)	820(6)	2800(10)	45	
O-32	−3439(7)	586(5)	3431(8)	70 *	
C-42	−3390(9)	503(7)	1950(10)	45	
O-42	−3062(6)	−40(4)	2157(7)	48 *	
C-52	−3084(9)	749(7)	1270(10)	51	
O-52	−3315(6)	1313(4)	1133(8)	58 *	
C-62	−3350(10)	484(9)	360(10)	76	
O-62	−4060(10)	474(7)	10(10)	121 *	
C-13	−3546(9)	−503(6)	1910(10)	45	
C-23	−3373(9)	−853(7)	2760(10)	48	
O-23	−3508(6)	−576(5)	3445(7)	61 *	
C-33	−2587(8)	−1052(6)	2990(10)	41	
O-33	−2442(6)	−1430(4)	3720(6)	55 *	
C-43	−2477(8)	−1352(7)	2200(10)	44	
O-43	−1719(5)	−1490(4)	2421(6)	42 *	
C-53	−2710(9)	−983(7)	1350(10)	50	
O-53	−3443(6)	−787(4)	1192(7)	49 *	
C-63	−2734(9)	−1298(7)	520(10)	54	
O-63	−3236(7)	−1737(5)	344(7)	69 *	
C-14	−1528(9)	−2025(7)	2290(10)	54	
C-24	−1003(8)	−2235(7)	3160(10)	52	
O-24	−1343(6)	−2227(5)	3837(7)	57*	
C-34	−278(8)	−1933(7)	3430(10)	50	
O-34	213(6)	−2154(5)	4228(7)	57 *	
C-44	25(8)	−1961(7)	2690(10)	50	
O-44	680(6)	−1626(4)	2925(7)	56 *	
C-54	−520(10)	−1739(8)	1860(10)	63	
O-54	−1169(6)	−2052(6)	1639(7)	71 *	
C-64	−260(20)	−1790(10)	1010(20)	115	
O-64(A)	−50(10)	−2270(10)	910(20)	141 *	0.72
O-64(B)	−840(30)	−1850(30)	230(40)	110	0.28
C-15	1359(9)	−1863(7)	2900(10)	55	
C-25	1943(9)	−1764(7)	3810(10)	53	
O-25	1723(6)	−1997(5)	4514(7)	63 *	
C-35	2070(9)	−1166(6)	3930(10)	44	
O-35	2648(6)	−1066(5)	4749(7)	64 *	

(Contd.)

Atom	x	y	z	U[a]	*K*
C-45	2284(9)	−930(6)	3170(10)	46	
O-45	2328(6)	−353(4)	3268(7)	57 *	
C-55	1710(10)	−1066(8)	2300(10)	64	
O-55	1569(6)	−1661(5)	2216(7)	64 *	
C-65	2000(10)	−970(10)	1470(20)	89	
O-65(A)	2630(10)	−1267(8)	1560(10)	95 *	0.72
O-65(B)	1400(40)	−950(30)	640(50)	136	0.28
C-16	2994(9)	−91(7)	3260(10)	56	
C-26	3260(10)	244(8)	4090(10)	62	
O-26	3414(6)	−64(5)	4875(7)	63 *	
C-36	2743(9)	712(7)	4110(10)	49	
O-36	3013(7)	1048(5)	4827(7)	65 *	
C-46	2594(9)	1049(6)	3240(10)	45	
O-46	2022(5)	1431(4)	3204(7)	46 *	
C-56	2340(10)	679(8)	2450(10)	77	
O-56	2853(7)	231(5)	2506(8)	81 *	
C-66	2310(10)	970(10)	1550(20)	104	
O-66(A)	3070(20)	1210(10)	1710(20)	203 *	0.63
O-66(B)	1890(30)	630(20)	780(30)	119	0.37
C-17	2158(9)	1983.9(0)	3120(10)	44	
C-27	1978(8)	2279(7)	3850(10)	47	
O-27	2435(6)	2103(4)	4709(7)	53 *	
C-37	1167(8)	2219(7)	3750(10)	48	
O-37	1004(6)	2523(5)	4445(7)	65 *	
C-47	710(8)	2417(7)	2860(10)	45	
O-47	−42(6)	2283(4)	2734(7)	50 *	
C-57	943(8)	2145(7)	2120(10)	47	
O-57	1715(6)	2186(4)	2290(7)	49 *	
C-67	580(10)	2395(8)	1190(10)	64	
O-67	694(7)	2958(6)	1195(8)	83 *	
W-61	−1240(20)	−3120(20)	660(30)	204 *	0.81
W-62(A)	0.0(0)	4626.6(0)	0.0(0)	98 *	0.50
W-62(B)	0.0(0)	−3560(20)	0.0(0)	309 *	0.50
W-62(C)	4320(20)	290(10)	1840(20)	248 *	1.00
W-65	4020(10)	−820(10)	1980(10)	149 *	1.00
W-67	−456(8)	3700(8)	806(9)	84 *	1.00
W-21	−890(10)	3700(10)	4770(10)	128 *	0.96
W-22	580(20)	−3250(20)	3950(20)	187 *	0.78
W-23	110(20)	4050(20)	3480(20)	215 *	0.86
W-24	−820(20)	−3240(20)	4650(20)	183 *	0.74
W-26	5120(40)	−120(30)	4570(40)	366 *	0.52
W-32	5150(20)	430(20)	3840(30)	370 *	0.77
W-W	4710(40)	980(30)	3050(40)	502 *	0.63
W	−610(50)	930(50)	4620(70)	553	0.66
N	−110(70)	90(50)	4180(60)	685	
C-1	−240(60)	320(50)	3120(60)	564	
C-2	−50(50)	40(40)	2240(50)	468	
C-3	−270(50)	310(40)	1300(50)	653	
C-5	50(60)	470(50)	580(60)	570	
C-4	−900(50)	−20(50)	640(60)	628	
C-6	−670(70)	760(50)	1650(80)	659	

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[a] Asterisks mark the anisotropic atoms $U = 1/3(U_{11} + U_{22} + U_{33})$.

TABLE II

Bond distances (Å) and angles (°) of the βCD–3,3-dimethylbutylamine inclusion complex

Bond[a]	*G-1*	*G-2*	*G-3*	*G-4*	*G-5*	*G-6*	*G-7*
βCD							
C-1–C-2	1.50(3)	1.48(3)	1.55(2)	1.52(2)	1.54(2)	1.49(2)	1.51(2)
C-1–O-5	1.41(2)	1.40(2)	1.40(2)	1.41(2)	1.37(2)	1.39(2)	1.40(2)
C-2–C-3	1.49(2)	1.55(2)	1.51(2)	1.51(2)	1.49(2)	1.53(2)	1.52(2)
C-2–O-2	1.39(2)	1.43(2)	1.37(2)	1.42(2)	1.43(2)	1.41(2)	1.43(2)
C-3–C-4	1.55(2)	1.50(2)	1.53(2)	1.47(2)	1.51(2)	1.56(2)	1.49(2)
C-3–O-3	1.46(2)	1.43(2)	1.44(2)	1.42(2)	1.43(2)	1.37(2)	1.45(2)
C-4–C-5	1.52(3)	1.52(3)	1.57(2)	1.50(2)	1.50(2)	1.50(2)	1.54(2)
C-4–O-4	1.44(2)	1.46(2)	1.42(2)	1.45(2)	1.42(2)	1.43(2)	1.43(2)
C-5–C-6	1.54(3)	1.51(3)	1.52(2)	1.57(4)	1.61(4)	1.58(3)	1.54(2)
C-5–O-5	1.43(2)	1.45(2)	1.43(2)	1.41(2)	1.49(2)	1.46(2)	1.42(2)
C-6–O-6(A)	1.31(3)	1.29(3)	1.41(2)	1.26(4)	1.38(3)	1.52(5)	1.40(2)
C-6–O-6(B)				1.38(6)	1.45(6)	1.50(5)	
O-4–C-1″	1.41(2)	1.44(2)	1.40(2)	1.44(2)	1.43(2)	1.40(1)	1.42(2)
3,3-Dimethylbutylamine							
C-1–N	1.71(8)						
C-1–C-2	1.70(7)						
C-2–C-3	1.56(7)						
C-3–C-4	1.55(9)						
C-3–C-5	1.52(7)						
C-3–C-6	1.55(8)						

Angle[a]	*G-1*	*G-2*	*G-3*	*G-4*	*G-5*	*G-6*	*G-7*
βCD							
C-1–C-2–C-3	108(1)	109(1)	108(1)	112(1)	108(1)	113(1)	110(1)
C-2–C-3–C-4	110(1)	108(1)	110(1)	108(1)	111(1)	108(1)	110(1)
C-3–C-4–C-5	108(1)	110(1)	111(1)	111(1)	110(1)	110(1)	111(1)
C-4–C-5–O-5	112(1)	108(1)	110(1)	109(1)	111(1)	112(1)	111(1)
C-5–O-5–C-1	112(1)	114(1)	114(1)	113(1)	112(1)	115(1)	116(1)
O-5–C-1–C-2	114(1)	111(1)	113(1)	108(1)	112(1)	111(1)	110(1)
C-1–C-2–O-2	112(1)	112(1)	112(1)	111(1)	111(1)	113(1)	112(1)
C-3–C-2–O-2	112(1)	109(1)	113(1)	112(1)	112(1)	110(1)	112(1)
C-2–C-3–O-3	112(1)	112(1)	109(1)	111(1)	109(1)	113(1)	109(1)
C-4–C-3–O-3	107(1)	109(1)	108(1)	112(1)	109(1)	110(1)	111(1)
C-4–C-5–C-6	112(2)	115(1)	112(1)	114(2)	113(1)	113(2)	114(1)
O-5–C-5–C-6	106(1)	106(1)	105(1)	104(1)	99(1)	104(2)	106(1)
C-5–C-6–O-6(A)	113(2)	114(2)	113(1)	112(2)	112(2)	105(2)	112(1)
C-5–C-6–O-6(B)				114(4)	122(3)	110(2)	
C-3–C-4–O-4	109(2)	106(1)	108(1)	108(1)	108(1)	107(1)	108(1)
C-5–C-4–O-4	111(1)	109(1)	109(1)	108(1)	109(1)	109(1)	109(1)
C-2–C-1–O-4′	109(2)	109(1)	105(1)	108(1)	107(1)	107(1)	107(1)
O-5–C-1–O-4′	110(1)	110(1)	111(1)	112(1)	112(1)	110(1)	111(1)
C-4–O-4–C-1″	118(1)	118(1)	119(1)	119(1)	118(1)	118(1)	119(1)
3,3-Dimethylbutylamine							
N–C-1–C-2	132(9)						
C-1–C-2–C-3	124(7)						
C-2–C-3–C-4	110(7)						
C-2–C-3–C-5	142(8)						
C-2–C-3–C-6	88(5)						
C-4–C-3–C-5	92(6)						
C-4–C-3–C-6	104(7)						
C-5–C-3–C-6	116(8)						

[a] Single primes denote the n − 1 residues; double primes denote the n + 1 residues.

TABLE III

Torsion angles[a] (Φ/°) of the βCD host

Angle	*G-1*	*G-2*	*G-3*	*G-4*	*G-5*	*G-6*	*G-7*
C-1–C-2–C-3–C-4	−55	−57	−55	−54	−56	−53	−56
C-2–C-3–C-4–C-5	56	57	55	56	55	52	52
C-3–C-4–C-5–O-5	−57	−58	−54	−60	−53	−54	−51
C-4–C-5–O-5–C-1	58	59	56	63	57	56	55
C-5–O-5–C-1–C-2	−58	−61	−60	−60	−59	−55	−58
O-5–C-1–C-2–C-3	57	58	57	55	59	55	57
O-4′–C-1–C-2–O-2	57	57	61	60	58	60	62
O-2–C-2–C-3–O-3	62	60	62	57	61	57	57
O-3–C-3–C-4–O-4	−62	−66	−66	−63	−65	−65	−67
O-4–C-4–C-5–C-6	66	69	70	66	77	72	70
C-4–C-5–C-6–O-6(A)	60	56	60	50	56	56	54
C-4–C-5–C-6–O-6(B)				149	−163	−167	
O-5–C-5–C-6–O-6(A)	−62	−63	−60	−68	−62	−66	−69
O-5–C-5–C-6–O-6(B)				30	79	72	
C-3–C-4–O-4–C-1″	130	128	130	126	127	126	128
C-5–C-4–O-4–C-1″	−114	−114	−109	−114	−113	−116	−111
C-4–O-4–C-1″–C-2″	−125	−129	−125	−126	−126	−128	−120
C-4–O-4–C-1″–O-5″	113	108	116	110	113	112	114

[a] Primes as in Table II

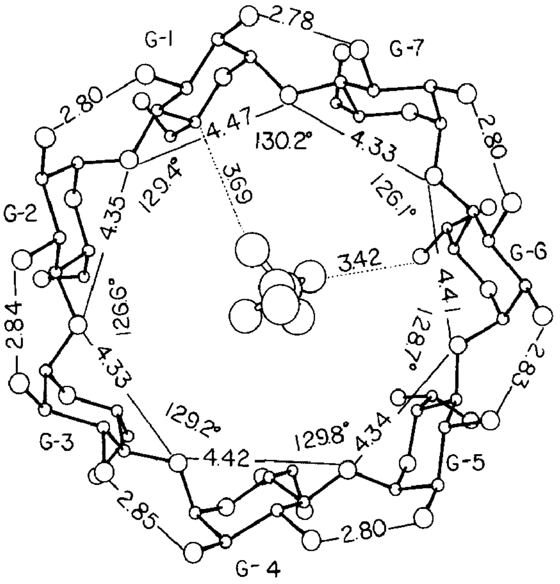

Fig. 2. O-4n···O-4(n+1), O-3n···O-2(n+1) distances and O-4(n−1)···O-4n···O-4(n+1) angles for the complex of βCD with 3,3-dimethylbutylamine. Intermolecular distances of <4.0 Å between host/guest are shown also.

TABLE IV

Parameters which describe the conformation of the βCD macrocyle

Residue	Φ[a]	d[b]	t[c]	a[d]
G-1	118	−0.033	10	82
G-2	118	0.045	13	81
G-3	119	−0.011	9	84
G-4	119	−0.016	12	80
G-5	118	−0.003	11	82
G-6	118	0.029	9	83
G-7	119	−0.012	6	86

[a] Glycosidic oxygen angle (°). [b] Deviations (Å) from the least-squares optimum plane of the seven O-4n atoms. [c] Dihedral angle (°) between the O-4n plane and the optimum plane through C-1n, C-4n, O-4n, and O-4′n. [d] Dihedral angle between the O-4n plane and the optimum plane through C-2n, C-3n, C-5n, and O-5n.

O-3n···O-2(n + 1) and O-3n···O-2(n + 1)–C-2(n + 1) were within the normal values for hydrogen bonds (average 116.7° and 118.2°, respectively).

The distances between the O-3 atoms of two adjacent βCD molecules indicate hydrogen bonding and dimer formation. Table V shows the intradimer H-bond distances O-3n···O-3′(8-n) and the corresponding angles. Distances between O-2n and O-3′(8-n) are in the range for very weak H-bonds 3.06–3.24 Å [average, 3.11(3) Å]. However, although the angles C-2n–O-2n···O-3′(8-n) [average, 114(1)°] could indicate H-bonding, the angles O-2n···O-3′(8-n)–C-3′(8-n) [average, 161(1)°] have high values. This means that O-2n atoms could be H-bond donors to O-3′(8-n), but O-3′(8-n) atoms do not have their lone pairs pointing towards the above H-atoms. By the same reasoning, there are no O-2···O-2′(8-n) H-bonds either, although some of these distances are ~3 Å.

The dimers form a C-centered layer parallel to the (*a*,*b*) plane (Fig. 3). The layers are stacked in such a way that the dimers form a channel along the *c* axis (Fig. 4) and are linked by H-bonds through water molecules. Direct H-bonding exists only between O-65(B) of one layer and O-64(B) of the layer above [O-65(B)···O-64(B) distance = 2.81 Å]. The relative shift between two consecutive dimers projected onto the (*a*,*b*) plane is 2.45(2) Å.

There are 10.7 water molecules per βCD molecule distributed over 14 molecular sites (denoted in Figs. 3 and 4 by W) and 12 are within H-bond distance from the oxygen atoms of the βCD hydroxyl groups. It is assumed that distances O···W of 2.50–3.06 Å and angles C–O···W of 98–144° indicate hydrogen bonding. Such wide ranges are generally accepted on account of the usual disorder of the water molecules and the inability to locate H-atoms in βCD complexes. The water sites have been labelled by the number of the closest oxygen atoms to which they are H-bonded. The H-bonds are listed in Table V. There are also hydrogen bonds between water molecules. One water molecule (W–W) is H-bonded only to other water molecules, and the last one (W) is entrapped inside the βCD dimer and hydrogen-bonds to the amino groups of the guests (Fig. 3 and Table V).

TABLE V

Intermolecular hydrogen bonds of the inclusion complex of βCD with 3,3-dimethylbutylamine

Intradimer

Residue	*Distance (Å)* *O-3n···O-3(8-n)*	*Angle (°)* *C-3n–O-3′n···O-3(8-n)*
G-1	2.81	115
G-2	2.87	117
G-3	2.74	117
G-4	2.82	119
G-5	2.74	117
G-6	2.87	119
G-7	2.81	119

With water molecules[a]

O-61	···W-61	2.87	C-61–O-61	···W-61	98
O-62	···W-62A	2.77	C-62–O-62	···W-62A	129
	···W-62B	3.00	C-62–O-62	···W-62B	123
	···W-62C	2.86	C-62–O-62	···W-62C	104
O-63	···W-67	2.80	C-63–O-63	···W-67	104
O-64(A)	···W-61	3.06	C-64–O-64(A)	···W-61	118
O-64(B)	···W-61	3.30	C-64–O-64(B)	···W-61	104
O-65	···W-65	2.78	C-65–O-65	···W-65	124
O-66	···W-62C	3.35	C-66–O-66	···W-62C	114
	···W-61	3.03	C-66–O-66	···W-61	141
O-67	···W-67	2.76	C-67–O-67	···W-67	123
O-21	···W-21	2.63	C-21–O-21	···W-21	112
O-22	···W-22	2.85	C-22–O-22	···W-22	102
O-23	···W-23	2.82	C-23–O-23	···W-23	105
O-24	···W-24	2.82	C-24–O-24	···W-24	100
O-26	···W-26	2.72	C-26–O-26	···W-26	103
O-32	···W-32	3.00	C-32–O-32	···W-32	144
O-34	···W-22	2.87	C-34–O-34	···W-22	110
O-35	···W-21	2.86	C-35–O-35	···W-21	117
O-36	···W-24	2.93	C-36–O-36	···W-24	112
N	···W	2.46	C-1–N	···W	93
N′	···W	2.84	C-1′–N′	···W	107

[a] Primes indicate atoms related by the screw axis.

Two water networks are formed, linking the primary and the secondary hydroxyl groups, respectively. None of the primary hydroxyl groups is linked to water of the secondary hydroxyl group and vice versa. The primary water network extends into an infinite sub-layer between two adjacent dimeric layers, whereas the secondary water network links the dimers of the same layer (Fig. 4).

The guest molecule is located inside the cavity, with the *tert*-butyl group buried in the hydrophobic region beneath the primary hydroxyl groups. The amino group is in the secondary-hydroxyl-group side but it is not H-bonded to any of them but to a water molecule entrapped inside the dimer interface (Table V).

TABLE I

Chemical shifts (δ) of proton resonances for chitosan in D_2O at 90° (pD 3)

Residue	*Proton*			
	H-1	*H-2*	*H-2/6*	*Acetyl-H*
GlcNAc (A)	4.55–4.65		3.5–4.0	2.04
GlcN (D)	4.85	3.15	3.5–4.0	

Identification of resonances in the spectrum. — The 300-MHz ^{1}H-n.m.r. spectrum of a fully *N*-deacetylated chitosan has been reported[15]. In the 500-MHz ^{1}H-n.m.r. spectrum (Fig. 1), five resonances (*a–e*) were identified (Table I). The resonances of H-1 and H-2 of a GlcN unit occur at 4.8 (*a*) and 3.1 p.p.m. (*e*), respectively. The identification of the resonance at 4.6 p.p.m. (*b*) as due to H-1 of a GlcNAc unit is based on the reported spectrum of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside[16]. These identifications were confirmed by a COSY experiment. The resonance at 2.04 p.p.m. (*e*) is due to the three NAc protons. No attempts were made to identify the resonances of H-2 of GlcNAc and H-3/6, which occur in the range 3.5–4.0 p.p.m.

Determination of the degree of N*-acetylation (d.a.).* — From the identification of the resonances (*a–e* in Fig. 1), it was possible to determine the d.a. In order to use all of the intensities of *a–e*, the following method was developed. Let *f* be defined as
(Total area of resonances)/(area of GlcNAc H-1 + NAc resonances)
$= (I_a + I_b + I_c + I_d + I_e)/(I_b + I_e)$.

If F_A is the mole fraction of *N*-acetylated units, then the mole fraction of *N*-deacetylated units is $1 - F_A$. Expressing *f* in terms of F_A gives
$f = (1 - F_A + F_A + 6 + 3F_A)/(F_A + 3F_A) = (7 + 3F_A)/4F_A$; [$I_c + I_d$ includes six protons from every monomer unit (GlcN or GlcNAc), and I_e is three times the mole fraction of the GlcNAc].

The mole fraction of *N*-acetylated units (F_A) may be expressed as
$F_A = 7(I_b + I_e)/[4(I_a + I_c + I_d) + I_b + I_e]$.

It is possible to determine the d.a. of chitosans at lower field (100 MHz), but not the diad frequencies. At 100 MHz, the d.a. can be determined either from the relative areas of the H-1 resonances or in combination with the area of the NAc resonances, as the HOD-signal (from the solvent) partly overlaps the resonances at 3.5–4.0 p.p.m.

Diad frequencies in homogeneous N*-deacetylated chitosans.* — In order to study the neighbouring-residue effect on the ^{1}H-n.m.r. resonances, a series of chitosan samples with degrees of *N*-deacetylation (d.d.) within the range from 51–96% (F_A 0.49–0.04) were prepared. Fractions of lower d.d. are insoluble in acidic media[2]. Figure 2 shows the region for the resonances of anomeric protons of these samples recorded at 500 MHz. The resonance of H-1 of a GlcNAc unit has two relatively well-resolved peaks that reflect two diad frequencies. These resonances suggest that the H-1 resonance of a GlcNAc unit is sensitive to a nearest neighbour, most probably to the following residue

The thermal parameters of the guest atoms have high values. An attempt to find more than one molecular site for the guest failed. Some bond lengths and angles differ from those expected for C–C and C–N single bonds (Table II). However, from the present structure as well as those of similar host/guest complexes involving CDs, it appears that guest disorder is an inherent phenomenon, as often occurs in the side chains of proteins. This disorder/flexibility in proteins is often a crucial factor for the manifestation of such biological phenomena as catalysis.

Several applications of inclusion complexes involve release rather than inclusion of the guest (pheromones, perfumes, *etc.*). It is likely that the release benefits from the lack of strong interactions that could immobilise the guest. The crystallographic information aims mainly at the determination of the orientation of the guest and its average position, rather than the precise position of all its atoms. It is the analysis of the structures of similar compounds[2] that may shed light on the host/guest interactions which govern the structure and properties of the inclusion complexes

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