Solid State and Solution Conformation of 6-{4-[*N*-*tert*-Butoxycarbonyl-*N*-(*N*′-ethyl)propanamide]imidazolyl}-6-deoxycyclomaltoheptaose: Evidence of Self-Inclusion of the Boc Group within the β-Cyclodextrin Cavity

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A new modified β-cyclodextrin (β-CD) derivative **1** that was functionalized in position 6 with Boc-Carcinine was synthesised and its crystal structure was determined. The structure reveals a "sleeping swan"-like shape, the covalently bonded Boc-Carcinine moiety forming a folded structure with the Boc group inserted within the hydrophobic cavity of the β-cyclodextrin. The conformation of the Carcinine moiety is determined by the inclusion of the Boc group and is further stabilised by three intramolecular hydrogen bonds, two between the amide N1–H group, the carbonyl C′1=O1 group and a primary hydroxylic group of the glucose unit 5, one between the carbonyl C′0=O0 group and the primary hydroxylic group of the glucose unit 2. The β-CD macrocycle differs only slightly from unmodified β-CDs, maintaining an approximate sevenfold symmetry. The solution structure of the new β-CD derivative was investigated by NMR spectroscopy and circular dichroism (c.d.) spectroscopy. In addition to a complete (^{1}H and ^{13}C) assignment of the pendant Boc-Carcinine group, the NMR study allowed the assignment of all the proton resonances associated with the β-CD macrocycle. Furthermore, NMR and c.d. results indicated that the self-inclusion of the Boc group within the β-CD cavity is retained in aqueous solution. In order to estimate the strength of this self-inclusion complex a series of competition experiments with the external guest 1-adamantanol was carried out using c.d. spectroscopy.

Introduction

Cyclodextrins (CDs) are cyclic oligomers of D(+)-glucopyranosyl units linked by α-1,4 glycosidic bonds. The most common CDs are α-, β-, and γ-cyclodextrin with six, seven, or eight glucose units, respectively.[1,2] These molecules are water-soluble and have the shape of a truncated cone with a hydrophobic cavity that allows the inclusion of various organic molecules in aqueous solution. They have been used successfully for a wide variety of applications including sensing molecules,[3] catalysis of chemical reactions,[4] enzyme models[5] and transport of pharmacologically active compounds.[6] The catalytic and complex formation abilities of CDs can be improved by the introduction of more reactive groups or additional binding sites into the macrocyclic ring. Several well-defined protocols for the chemical modification of CDs are available, allowing the synthesis of a great number of substituted CDs.[7] However, only with a detailed understanding of the structure-function relationships can the binding properties and basic phenomena governing the inclusion process be investigated. Since it is not always possible to obtain suitable crystals of modified cyclodextrins, very few crystal structures have been reported.[8] In the absence of solid state structural information, NMR spectroscopy represents a powerful tool for the analysis of modified cyclodextrins.[9] However, selectively substituted CDs have complicated NMR spectra, and although advanced two-dimensional[9,10] and selective experiments[11] are now widely used for the structure determination in solution, the detailed structure of modified CDs including the conformation of the pendant groups was determined in only a few cases.[8c,12] In the present paper, we report the synthesis, crystal structure and a detailed spectroscopic (NMR and c.d.) study of the new 6-{4-[*N*-*tert*-butoxycarbonyl-*N*-(*N*′-ethyl)propanamide]imidazolyl}-6-deoxycyclomaltoheptaose or β-CD-Carc-Boc (**1**) in aqueous solution (Scheme 1). In this molecule a Boc-carcinine (*N*-*tert*-butoxycarbonyl-β-alanylhistamine) moiety is attached to the β-CD at position 6. It is an intermediate for the synthesis of carcinine β-CD derivatives, a reaction we are currently working on. Carcinine is an imidazole peptide-type molecule of biological interest.[13] The present study provides direct evidence for the intramolecular inclusion of the Boc group into the β-CD cavity both in the solid state and in aqueous solution. Moreover, competition experiments with external 1-adamantanol monitored by circular dichroism (c.d.) spectroscopy allowed an evaluation of the strength of this self-inclusion complex.

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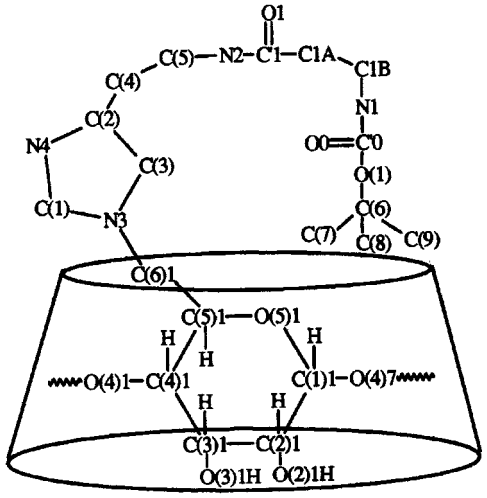


Scheme 1. General formula of β-CD-Boc-carcinine; the cyclodextrin atoms are indicated as C(*m*)*n* and O(*m*)*n*, where m denotes the m^{th} atom within the n^{th} glycosidic residue Gn

Results and Discussion

Crystal Structure of 1

A stereo view of the molecular structure is presented in Figure 1. The bond lengths, and the bond and torsion angles observed for the β-CD molecule and the covalently attached functional group are unexceptional. A selection of torsion angles which define the linkage bonds between the glucose units and the orientation of the primary hydroxyl groups is shown in Table 1a. The Cremer and Pople puckering parameters of the glucose units,[14] which are very close to the usual $^4C_1$ chair conformation, are listed in Table 2. The total puckering amplitude Q for all residues (0.56–0.58 Å) is slightly lower than for an ideal cyclohexane chair conformation (0.63 Å), whereas the parameter which measures the magnitude of ring distortion is in the range 2.6°– 6.4°. All primary hydroxyl groups assume a gauche$^+$/gauche$^-$ orientation and point away from the macrocycle cavity, except the ones from the glucose units 2, 5 and one of the statistical bonds from unit 3 [O(6)3A atom]. As for the latter, the C(6)–O(6) bond is trans to the C(4)–C(5) bond and gauche$^+$ to the O(5)–C(5) bond; consequently, these hydroxyl groups point into the cavity.

The β-CD macrocycle differs only slightly from the hydrated uncomplexed or methylated β-CDs,[15–17] maintaining an approximate sevenfold symmetry; geometrical data are listed in Table 3. The glucosidic O(4) atoms form a heptagon with radii in the range of 4.91–5.08 Å and side lengths of 4.27–4.42 Å.

The O(4) atoms are nearly coplanar: a maximum deviation from the least-squares plane of 0.31 Å is observed for the O(4) atom of unit 3. The dihedral angles between the O(4) plane and the least-squares plane through O(4)*n*+1, C(1)*n*, C(4)*n*, and O(4)*n* lie in the range 2.1–25.4°. The round shape of the β-CD ring is stabilised by intramolecular H-bonds between the secondary hydroxyl groups of neighbouring glucose residues: O(2)*n*···O(3)*n*–1. Hydrogen-bond lengths lie in the usual range (2.76–2.98 Å) found in β-CDs.

The structure reveals a "sleeping swan"-like shape, the covalently bonded Boc-carcinine moiety being folded with the Boc group inserted into the hydrophobic cavity of the β-CD ring. This structure is similar in dimensions and conformation to structures we previously reported for β-CDs monosubstituted with the cyclic dipeptide c (L-histidyl-L-leucyl) (cHL-βCD, Figure 2) and the Boc-amino-ethyl-imidazolyl moiety (Cdmhboc).[8b][8e] With respect to the β-CD macrocycle, the functional groups assume the same type of folded conformation, indicating the existence of similar host-guest binding forces between these groups and the hydrophobic cavity. The conformation of the Boc-carcinine moiety is best described by the torsion angles given in Table 1b. The analysis of the dihedral angles between the

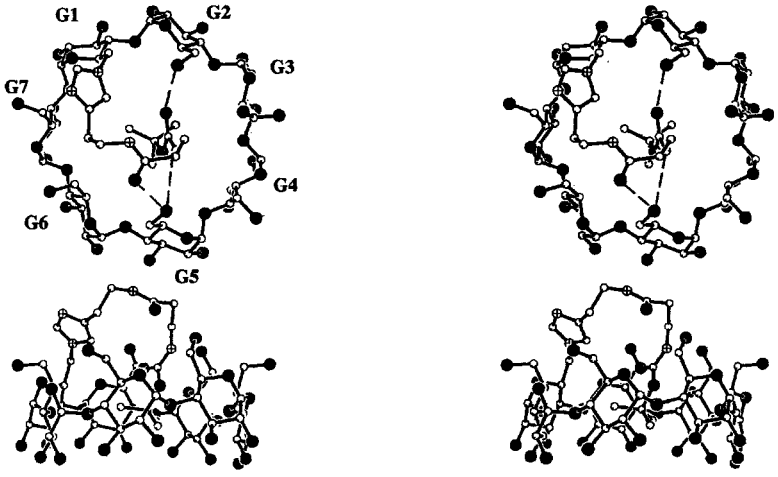


Figure 1. Stereo view of the β-CD-Boc-carcinine molecular model; G1–7 refer to the glucose units

Table 1. Selected torsion angles (°) describing a) the linkage bonds between the glucose residues and the orientations of the primary hydroxy groups; b) the conformation of the Boc-carcinine moiety

		G1	G2	G3	G4	G5	G6	G7
a)	C(3)*n*–C(4)*n*–O(4)*n*–C(1)*n*+1	124.5	122.4	122.7	139.3	126.1	118.9	139.9
	C(5)*n*–C(4)*n*–O(4)*n*–C(1)*n*+1	–114.7	–118.7	–116.6	–100.2	–112.0	–119.7	–100.0
	O(5)*n*–C(1)*n*–O(4)*n*–1–C(4)*n*–1	114.0	107.7	108.5	104.4	117.4	105.0	99.6
	C(2)*n*–C(1)*n*–O(4)*n*–1–C(4)*n*–1	–124.4	–130.5	–129.4	–134.6	–122.4	–133.3	–139.8
	C(4)*n*–C(5)*n*–C(6)*n*–O(6)*n*		–162.7	–171.6 60.7	58.2	177.6	49.6	55.4
	O(5)*n*–C(5)*n*–C(6)*n*–O(6)*n*		76.5	67.7 –60.0	–61.7	58.9	–70.8	–66.4
		Angle (°)			Angle (°)			
b)	O(5)1–C(5)1–C(6)1–N3	59.5		C(2)–C(4)–C(5)–N2	–71.0			
	C(4)1–C(5)1–C(6)1–N3	178.4		C(4)–C(5)–N2–C′1	–74.1			
	C(5)1–C(6)1–N3I–C(1)	–99.6		C(5)–N2–C′1–C1A	179.4			
	C(5)1–C(6)1–N3I–C(3)	75.2		N2–C′1–C1A–C1B	–86.6			
	C(6)1–N3–C(3)–C(2)	–175.5		C′1–C1A–C1B–N1	–57.6			
	C(6)1–N3–C(1)–N4	176.3		C1A–C1B–N1–C′0	140.7			
	N3–C(3)–C(2)–C(4)	174.8		C1B–N1–C′0–O(1)	–177.7			
	N3–C(1)–N4–C(2)	–1.0		N1–C′0–O(1)–C(6)	–174.9			
	C(3)–C(2)–C(4)–C(5)	103.5		C′0–O(1)–C(6)–C(7)	59.0			
	C(1)–N4–C(2)–C(4)	–174.9		C′0–O(1)–C(6)–C(8)	–69.7			
	N4–C(2)–C(4)–C(5)	–81.4		C′0–O(1)–C(6)–C(9)	174.9			

Table 2. Puckering parameters of glucose units according to ref.[14]

	G1	G2	G3	G4	G5	G6	G7
Q (Å)	0.57	0.56	0.56	0.56	0.56	0.58	0.56
θ (°)	4.52	4.29	2.61	5.31	6.42	2.64	2.94

the C(2)–C(4)–C(5)–N2 and C(4)–C(5)–N2–C′1 torsion angles (–71°, –74°, respectively) are close to the ideal gauche$^-$ (–60°) conformation. Thus, the terminal Boc group enters the centre of the cavity from the side of the primary hydroxyl groups and is buried inside the β-CD macrocycle. Therefore, favourable intramolecular host-guest interactions occur with the hydrophobic cavity. The centre of gravity of the *tert*-butyl group is positioned 0.20 Å below the O(4) plane on the same side as the secondary hydroxyl groups. The C(7) and C(9) methyl groups are 0.48 and 0.72 Å away from the O(4) plane and pointing in the same direc-

Table 3. Geometrical data

Residue	Radius (Å)[a]	Distance (Å)[b]	Angle (°)[c]	Tilt angle[d]	Planarity[e]
G1	5.07	4.39	128.3	19.7	–0.22
G2	5.04	4.37	127.5	18.3	0.07
G3	4.94	4.42	130.0	10.7	–0.31
G4	5.08	4.27	127.1	5.6	0.11
G5	5.07	4.42	126.8	25.4	0.25
G6	4.91	4.34	131.2	21.4	–0.24
G7	5.07	4.36	126.8	2.1	–0.09

[a] The radius is measured from the centre of gravity of the seven O(4) atoms to each O(4) atom. – [b] The distance is defined as the $O(4)_n$–$O(4)_{n+1}$ distance. – [c] The angle is defined as the $O(4)_{n-1}$–$O(4)_n$–$O(4)_{n+1}$ angle. – [d] The tilt angle is defined as the angle between the O(4) atoms plane and the plane formed by $O(4)_{n+1}$, $C(1)_n$, $C(4)_n$, $O(4)_n$ of each glucose residue. – [e] Planarity is defined as the $O(4)_n$ distance from the O(4) atoms plane.

imidazole ring and the O(4) atoms plane (44.2°) as well as between the –CO–NH– group and the O(4) atoms plane (30.7°) indicates a bent conformation involving the C(4)–C(5) and N2–C(5) bonds. In fact, the observed values for

Figure 2. Stereo view of the superimposed β-CD-Boc-carcinine, β-CDc(L-histidyl-L-leucyl),[8e] and β-CD-Boc-amino-ethyl-imidazolyl[8b]

tion. The C(8) is removed form this plane by 0.25 Å and on the side of the primary hydroxyl groups. This arrangement of the *tert*-butyl group is similar to that observed for Boc-amino-ethyl-imidazolyl, in spite of different sizes, flexibilities, and conformational parameters (C(2)–C(4)–C(5)–N2 and C(4)–C(5)–N2–C′1 torsion angles *g*-, *g*- for β-CD-Boc-carcinine and *g*-, *trans* for Cdmhboc) of the two functional groups (Figure 2). In addition to van der Waals interactions between the hydrophobic *tert*-butyl group and the β-CD cavity, the folded conformation of the Boc-carcinine moiety is stabilised by three intramolecular H-bonds involving the amide N1–H group, the carbonyl C′1=O1 group and a primary hydroxyl group of the glucose unit 5 as well as the carbonyl C′0=O0 group and the primary hydroxyl group of the glucose unit 2 (Figure 1). A comparative analysis of β-CD structures with larger covalently bonded functional groups shows that the intramolecular H-bonds between these moieties and the primary hydroxyl groups on the top of the β-CD rim are a common feature. It could be one of the driving forces for folding the covalently bonded moieties, leading to the self-inclusion into the cyclodextrin cavity. In the crystal structure, one of the water molecules (Ow1) is H-bonded to the N4 of the imidazolyl ring. This further stabilises the conformation of the substituted β-CD.

In Figure 3, the mode of packing of the functionalized β-CD molecules is shown along the *b* axis. Several direct intermolecular hydrogen bonds form links between primary and secondary hydroxyl groups of symmetry related units (Scheme 2). The substituted β-CD molecules form columns related by a 2_1 screw axis. They are inclined with respect to the axis by about 30°, yielding a herringbone packing mode that is typical for monomeric β-CD complexes.[8c,8e,18,19] Within each layer, the β-CD macrocycles are held together by a complicated intermolecular hydrogen bond network of numerous water molecules and hydroxyl groups (Table 4). Of the eight water molecules, Ow1, Ow2, Ow3 and Ow4 link together two-fold symmetry related β-CD molecules. All water molecules are hydrogen bonded to neighbouring primary and/or secondary hydroxyl groups of symmetry related β-CD molecules, giving rise to a network of hydrogen bonds in the crystal.

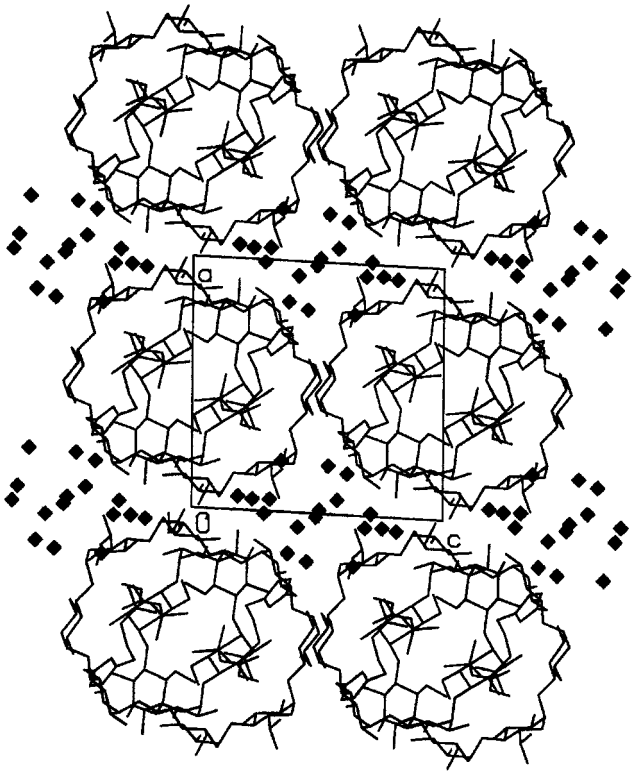
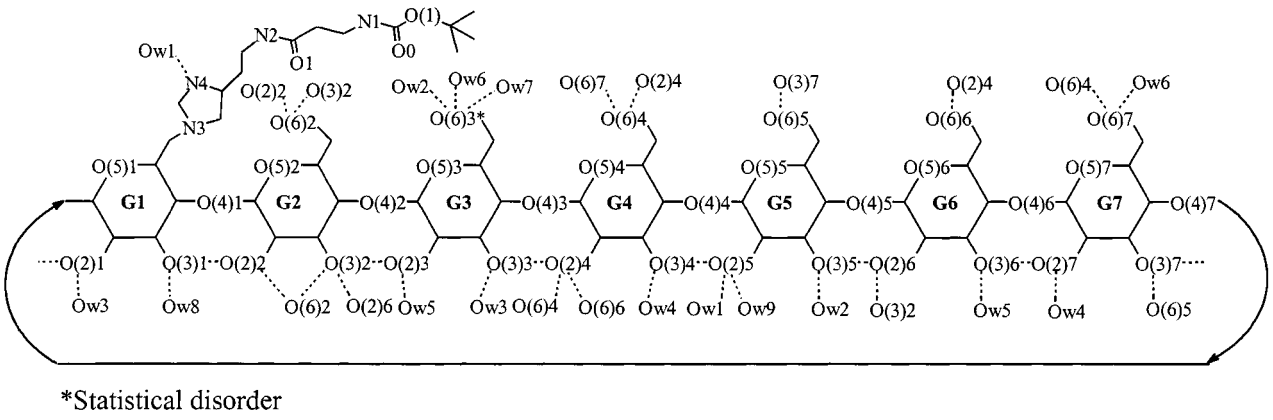


Figure 3. Crystal packing of the β-CD-Boc-carcinine viewed down the crystallographic *b* axis

Table 4. Inter- and intramolecular H-bonds; a) $O(2)_n$–$O(3)_{n-1}$ distances; b) intra- and intermolecular β-CD interactions; c) interactions between β-CD and water molecules; d) Water-water interactions

		Distance (Å)	
a) O2(1)	O(3)7	2.985	
O(2)2	O(3)1	2.834	
O(2)3	O(3)2	2.757	
O(2)4	O(3)3	2.832	
O(2)5	O(3)4	2.924	
O(2)6	O(3)5	2.923	
O(2)7	O(3)6	2.877	
		Distance (Å)	
b) O0	O(6)2	2.77	*x*, *y*, *z*
O1	O(6)5	2.73	*x*, *y*, *z*
N1	O(6)5	3.15	*x*, *y*, *z*
O(2)2	O(6)2	2.68	1 – *x*, –1/2 + *y*, 1 – *z*
O(3)2	O(2)6	2.90	1 – *x*, –1/2 + *y*, –*z*
O(3)2	O(6)2	3.27	1 – *x*, –1/2 + *y*, 1 – *z*
O(2)4	O(6)4	2.77	–*x*, –1/2 + *y*, –*z*
O(2)4	O(6)6	3.12	1 – *x*, –1/2 + *y*, –*z*
O(6)4	O(6)7	2.87	–1 + *x*, *y*, *z*
O(6)5	O(3)7	2.90	1 – *x*, 1/2 + *y*, –*z*
		Distance (Å)	**Symmetry**
c) N4	Ow1	2.79	1 – *x*, 1/2 + *y*, 1 – *z*
O(2)1	Ow3	2.78	1 + *x*, *y*, *z*
O(3)1	Ow8	3.00	1 + *x*, *y*, *z*
O(2)3	Ow5	2.63	1 – *x*, –1/2 + *y*, 1 – *z*
O(3)3	Ow3	2.76	*x*, *y*, *z*
O(6)3a	Ow2	2.80	*x*, *y*, *z*
O(6)3a	Ow6	2.90	*x*, *y*, *z*
O(6)3b	Ow7	2.88	*x*, *y*, *z*
O(3)4	Ow4	2.82	–*x*, –1/2 + *y*, –*z*
O(2)5	Ow1	2.72	*x*, *y*, –1 + *z*
O(2)5	Ow9	2.96	–*x*, 1/2 + *y*, –*z*
O(3)5	Ow2	2.92	*x*, *y*, –1 + *z*
O(3)6	Ow5	2.76	*x*, *y*, –1 + *z*
O(2)7	Ow4	2.78	1 – *x*, –1/2 + *y*, –*z*
O(6)7	Ow6	2.24	1 – *x*, *y*, *z*
		Distance (Å)	**Symmetry**
d) Ow1	Ow7	2.79	*x*, *y*, *z*
Ow1	Ow2	3.01	*x*, *y*, *z*
Ow2	Ow8	2.88	–*x*, 1/2+*y*, 1–*z*
Ow3	Ow8	2.19	*x*, *y*, *z*
Ow3	Ow9	2.77	*x*, *y*, *z*
Ow4	Ow6	2.93	*x*, *y*, *z*
Ow4	Ow9	2.87	*x*, 1+*y*, *z*
Ow5	Ow9	2.87	1–*x*, 1/2+*y*, 1–*z*



Scheme 2. Intermolecular hydrogen-bond scheme in β-CD-Boc-carcinine

Spectroscopic Studies in Aqueous Solution

Unsymmetrically modified β-CDs usually give rise to very complicated ^{1}H NMR spectra. The proton assignment becomes even more complex in the case of strong interactions between the pendant moiety and the β-CD, resulting in considerable perturbation of the axial symmetry of the β-CD macrocycle with different spreading and overlapping of most of the resonances associated with each glucopyranoside ring (Figure 4). On the other hand, proton-decoupled ^{13}C NMR spectra (Figure 5) usually show that the carbon atoms resonate in spectral regions where the atoms of unmodified β-CD are also found. Therefore, heteronuclear correlated two dimensional NMR experiments can be a valuable aid for the assignment of the proton resonances. By using this approach, we investigated, if the solid state

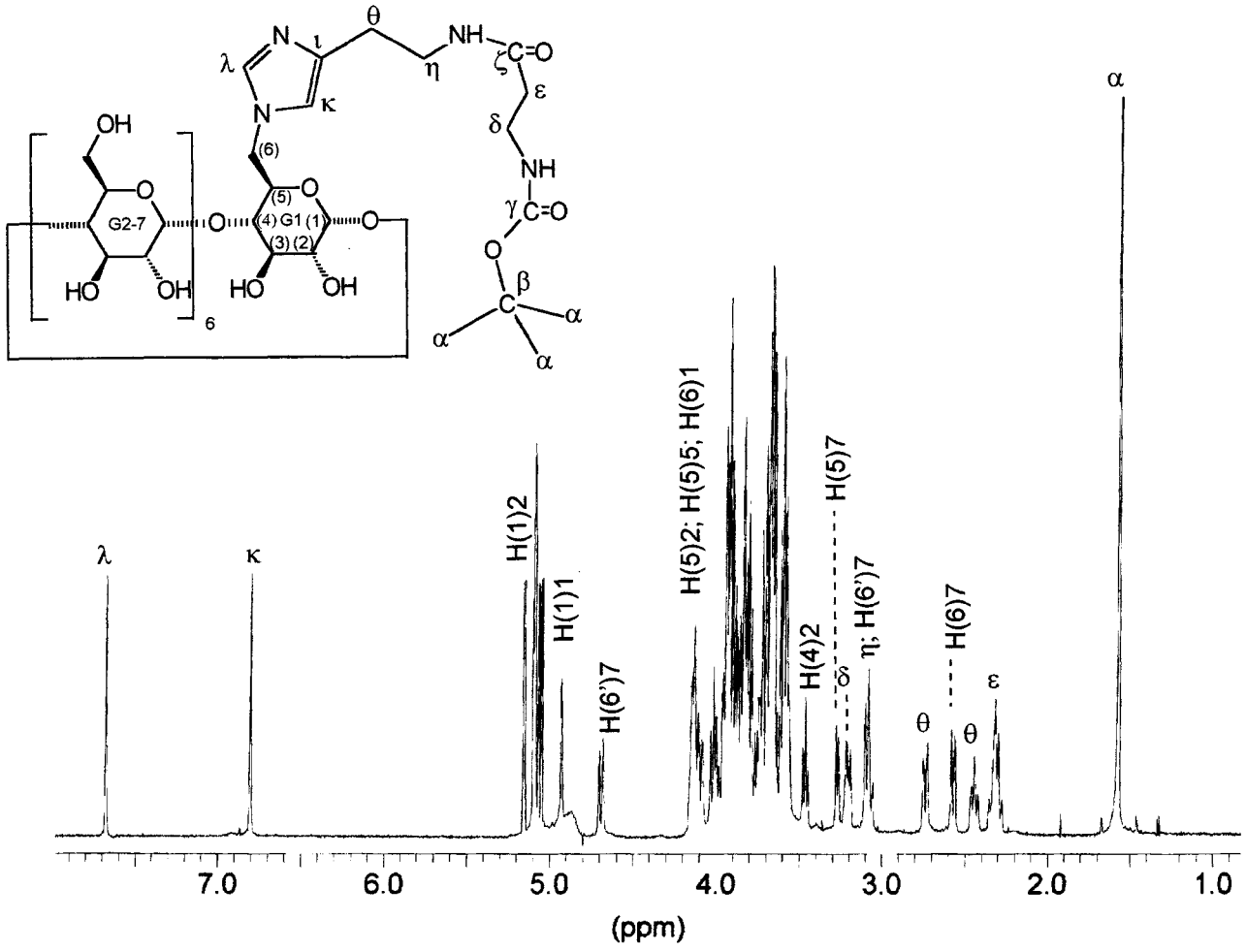


Figure 4. ^{1}H NMR spectrum (600 MHz) of **1** in D_2O with partial assignments (for complete assignment see Tables 5 and 6) and the schematic structure of **1** with the labelling system used in the assignment of NMR signals

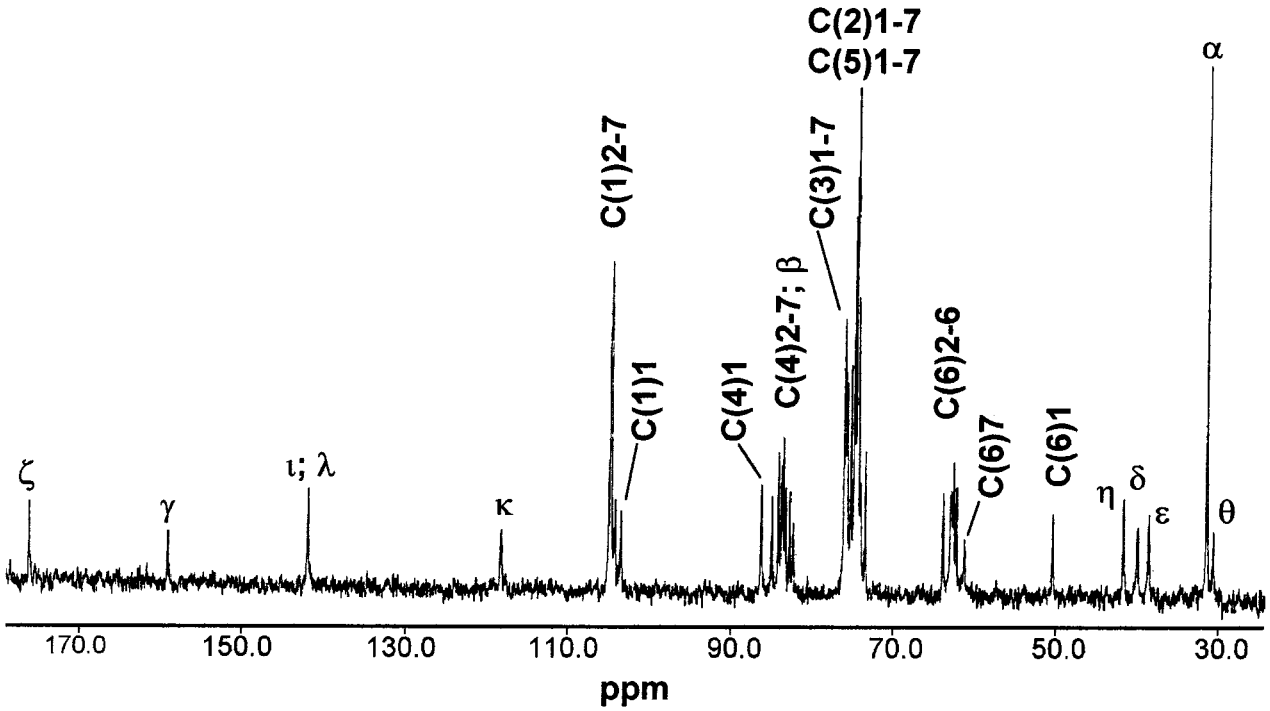


Figure 5. $^{13}C\{^1H\}$ NMR spectrum (62.5 MHz) of **1** in D_2O

conformation of β-CD-Carc-Boc (**1**) was also retained in aqueous solution. The NMR characterisation of **1** was achieved by the combined analysis of homonuclear (DQF-COSY,[20] TOCSY,[21] ROESY[22]) and heteronuclear (HSQC,[23] HMBC[24]) 2D NMR spectra:

a) Intraglucoside connectivities: the combination of COSY and TOCSY spectra allowed the assignment of the proton signals of each glucopyranose ring. The analysis was also aided by the HSQC spectrum which provided useful information for the identification of the proton positions within the glucopyranoside ring.

b) Determination of interglucoside connectivities: the sequence by which the glucose units are connected was obtained by analysing the ROESY spectrum. The NOE cross-peaks between H(1) and H(4) protons in adjacent units revealed the connecting sequence of the glucosides. In Table 5 the assignment of the β-cyclodextrin protons is reported.

Table 5. ^1H NMR data (recorded at 600 MHz in D_2O. Chemical shifts are δ values and refer to the residual water peak at δ = 4.8) for β-CD-Carc-Boc (**1**). Figure 4 shows the numbering system used for the assignment.

Position	H(1)	H(2)	H(3)	H(4)	H(5)	H(6)
G1	4.93	3.72	3.86	3.60	4.02	4.16, 4.71
G2	5.16	3.74	3.96	3.48	4.18	3.77, 3.97
G3	5.09	3.70	3.94	3.67	3.68	3.70, 3.85
G4	5.07	3.67	3.98[a]	3.67	3.91[a]	3.96, 4.12
G5	5.10	3.70	3.83	3.61	4.16	3.85, 4.04
G6	5.11	3.70	3.91	3.62	3.75	3.77, 3.96
G7	5.05	3.65	3.83	3.59	3.29	2.60, 3.11

[a] Interchangeable values.

c) Assignment of the pendant moiety: the complete ^1H and ^{13}C assignment of the signals associated with the Boc-carcinine moiety was accomplished by the combined analysis of COSY, HSQC and HMBC spectra. In particular, the HMBC spectrum allowed the unambiguous assignment of the four methylene groups of the carcinine and the identification of the quaternary carbon of the *tert*-butyl group which resonates in the same region as the C(4) carbons. Table 6 shows the complete assignment of the Boc-Carcinine moiety.

Table 6. Boc-Carcinine assignments obtained from DQF COSY, HSQC and HMBC at 600 MHz in D_2O. Chemical shifts are δ values and refer to the residual water peak at δ = 4.8. Figure 4 shows the numbering system used for the assignment.

Position	δ_H	δ_C
α	1.57	31.4
β		82.5
γ		159.2
δ	3.24, 3.61	39.9
ε	2.34	38.5
ζ		176.3
η	3.10, 3.70	41.6
θ	2.47, 2.75	30.6
ι		142.0
κ	6.82	118.2
λ	7.71	142.0

d) Analysis of the solution conformation: if the Boc moiety is included in the CD cavity, NOE correlations between the protons of the Boc group and the protons H(3), H(5), or H(6) of the β-CD moiety should be observed. Indeed,

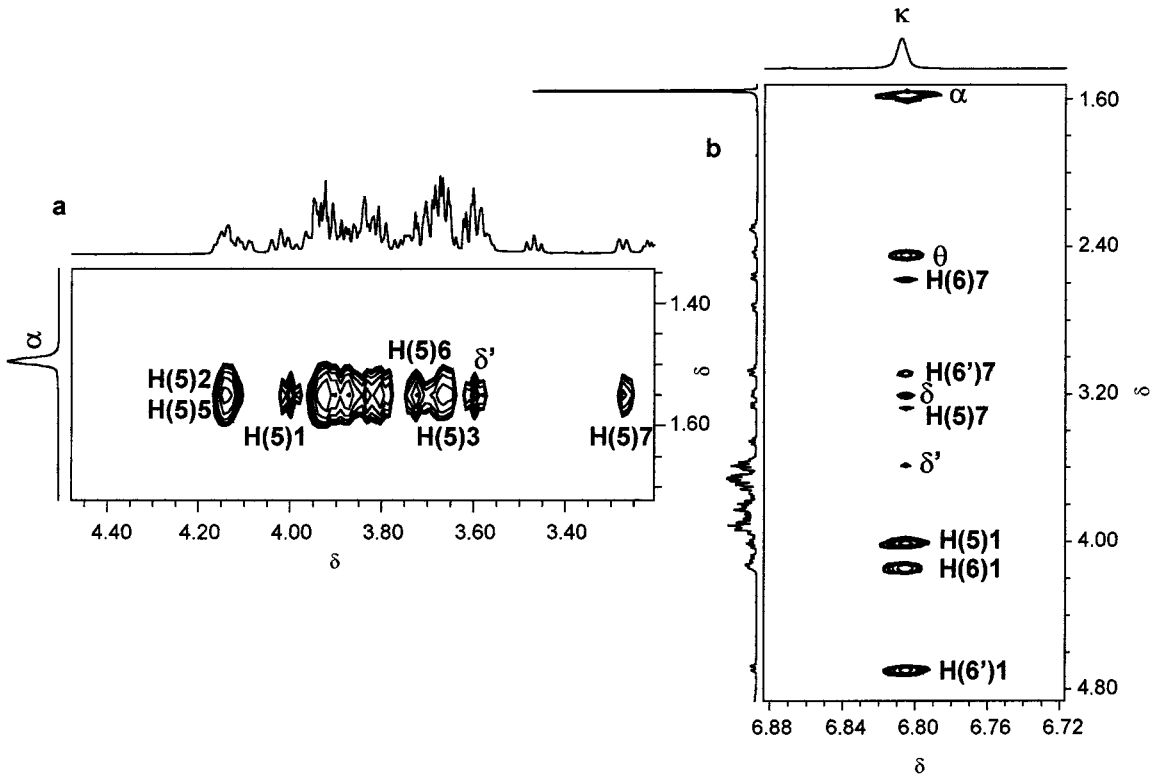


Figure 6. Portions of the 600 MHz ROESY spectrum of **1** in D_2O

the ROESY spectrum showed a series of NOE connectivities between the singlet α (δ = 1.57) and several protons of the cyclodextrin moiety. By careful analysis, it was possible to assign the majority of NOE cross-peaks to H(5) protons. Those of the H(3) protons overlapped completely, due to their narrow spectral dispersion (Figure 6a). This is mainly the result of both the aliphatic nature of the Boc group and the magnetic equivalence of its three methyl groups. This causes the NOE contacts to appear as a strip with several correlations overlapped.

On the other hand, the ROESY spectrum provided useful information concerning the conformation of the pendant moiety: the imidazole κ-proton (δ = 6.82) showed NOE connectivities with the *t*-butyl singlet α (δ = 1.57), one proton of the θ-methylene (δ = 2.47) and, to a lesser extent, with the δ-methylene (δ = 3.24, 3.61) (Figure 6b). This indicates a folded conformation of the Boc-carcinine pendant. The same imidazole proton showed NOE contacts to the H(5)1, H(6)1, H(6′)1, H(5)7, H(6)7, and H(6′)7 protons (Figure 6b), suggesting that the imidazole ring is placed almost perpendicular to the cavity of the β-cyclodextrin. At the same time, it is turned toward the H(5)7, H(6)7, H(6′)7 protons which, as a consequence of the shielding effect of the imidazole ring, experience an upfield shift (Table 5). This kind of orientation is not new and has been observed in other β-cyclodextrin derivatives in which the imidazole group is directly linked to the C(6) carbon.[8b,25] Although the NOE correlations between the protons of the Boc group and those of the β-cyclodextrin moiety were also observed in an intermolecular complex in which the Boc group is included in the cavity of another cyclodextrin, the NOE correlations observed within the Boc-carcinine pendant can be explained only by a self-inclusion complex. Moreover, the observed diastereotopicity of the δ, η, and θ methylenes (Table 6) suggest a certain rigidness of this part of the molecule and is in agreement with the hypothesis of the self-inclusion of the Boc group. ROESY experiments carried out at various concentrations did not show substantial differences and therefore reinforce the hypothesis of the self-inclusion.

The data above suggest that several effects were generated by the inclusion of the Boc group into the β-cyclodextrin cavity. Since the pendant was non aromatic the H(3) protons were little dispersed (Δδ = 0.15). In contrast, a greater dispersion was observed for the H(5) (Δδ = 0.89) and H(6) (Δδ = 2.11) protons. This can be explained by the anisotropy shielding effect of the carbonyl groups and the imidazolyl ring which, being closer to the upper rim of the macrocycle, influence the H(5) and H(6) protons. The H(3) protons are located in the lower part of the cavity and are therefore less affected. The remaining H(1), H(2) and H(4) protons, which are located outside the cavity, should be little affected by the inclusion; however, while this is true for H(2) (Δδ = 0.09) and H(4) (Δδ = 0.09; with the exception of the H(4)2 proton that resonates at δ = 3.48) the resonances associated with the anomeric protons H(1) (Δδ = 0.23) are almost completely separated (Figure 4). If self-inclusion of a pendant group occurs, the resonances of

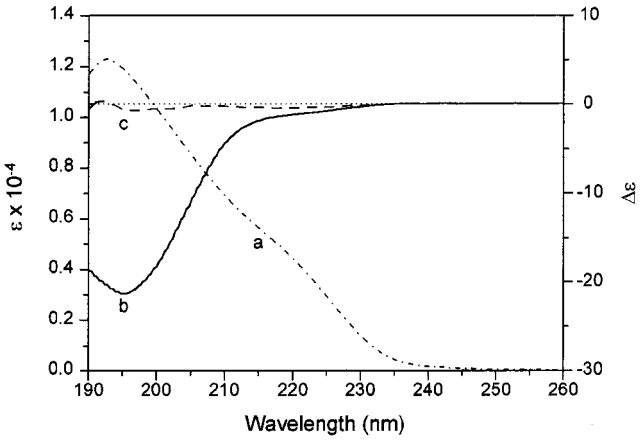


Figure 7. Absorption (**a**) and circular dichroism (**b**) spectra of **1** in H_2O at pH 7; (**c**) circular dichroism spectrum (pH 7) of the parent free amine β-CD-carcinine derived from **1** after cleavage of the Boc group

the anomeric protons are generally dispersed and indicate the reduction of the sevenfold symmetry of the modified β-cyclodextrin macrocycle.[12a,26] A further observation concerns the carbon resonances. Spreading ^{13}C NMR signals are mainly believed to reflect complexation induced conformational changes in cyclodextrins.[27] In the ^{13}C spectrum of **1**, most of the β-cyclodextrin carbons are distinctively inequivalent (Figure 5). Moreover, the C(4) carbons are clearly visible as seven peaks. This particularly strong effect can be attributed to the inclusion of the Boc group which causes the distortion of one of the torsion angles involved in the glycosidic bond.[28]

Further evidence for the self-inclusion of the Boc group was provided by c.d spectroscopy. It is known that an achiral guest molecule in the chiral cyclodextrin cavity may exhibit an induced circular dichroism (I.c.d.) in its absorption regions.[29] The UV and c.d. spectra of **1** are reported in Figure 7. The c.d. spectrum exhibits a strong negative band at ca. 195 nm; this signal which almost coincides with the absorption band observed in the UV spectrum (λ_{max} = 192 nm). Moreover, we observed that the cleavage of the Boc group by TFA, which produces the free-amine derivative, causes almost complete disappearance of the c.d. signal (Figure 7). This indicates that the signal observed in the c.d. spectrum of **1** is related to the Boc group and is consistent with the inclusion of the Boc group into the β-cyclodextrin cavity. Concentration-dependent c.d. studies were carried out in water at 25 °C and pH 7 in the range of 8–200 μM. The c.d. spectrum was not affected by this concentration change, indicating the intramolecular nature of the inclusion complex (not shown).

The effect of a varying pH on the c.d. spectrum of **1** is reported in Figure 8. The data indicates that the ellipticity at 195 nm is increased by an increasing pH. A clear isodichroic point is observed near 210 nm. A plot of $-\Delta\varepsilon_{195}$ against the pH values gives a sigmoidal curve which shows a strong increment of the ellipticity in the range of pH 4–7 (Figure 8). Since the pK_a of the imidazolyl moiety is expected to occur in this pH range, it is reasonable to hypothesise that its protonation state influences the self-inclusion process of the Boc group into the β-cyclodextrin cavity.

In order to verify this hypothesis, a series of c.d. experiments were carried out in the presence of 1-adamantanol. The spectra were collected at pH 4.1 and 7.1. The obtained data (Figure 9) display a similar trend indicating a decrease in intensity for the negative dichroic band at 195 nm only at high excess of the competitive guest. This suggests that the Boc group is held tightly within the β-CD cavity. However, at pH 4.1, only a minor excess of 1-adamantanol is needed to observe a significant decrease of the signal at 195 nm, suggesting that at this pH the expulsion of the Boc group from the cavity is less difficult. On the basis of these data, we hypothesise that the imidazole ring acts as a trigger

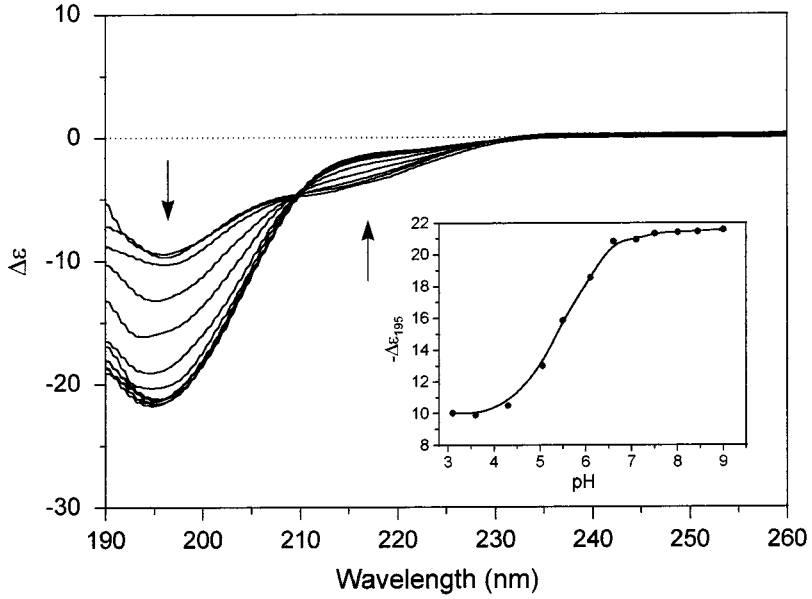


Figure 8. Circular dichroism spectra of **1** in H_2O at different pH values; inset: plot of -Δε at 195 nm vs pH

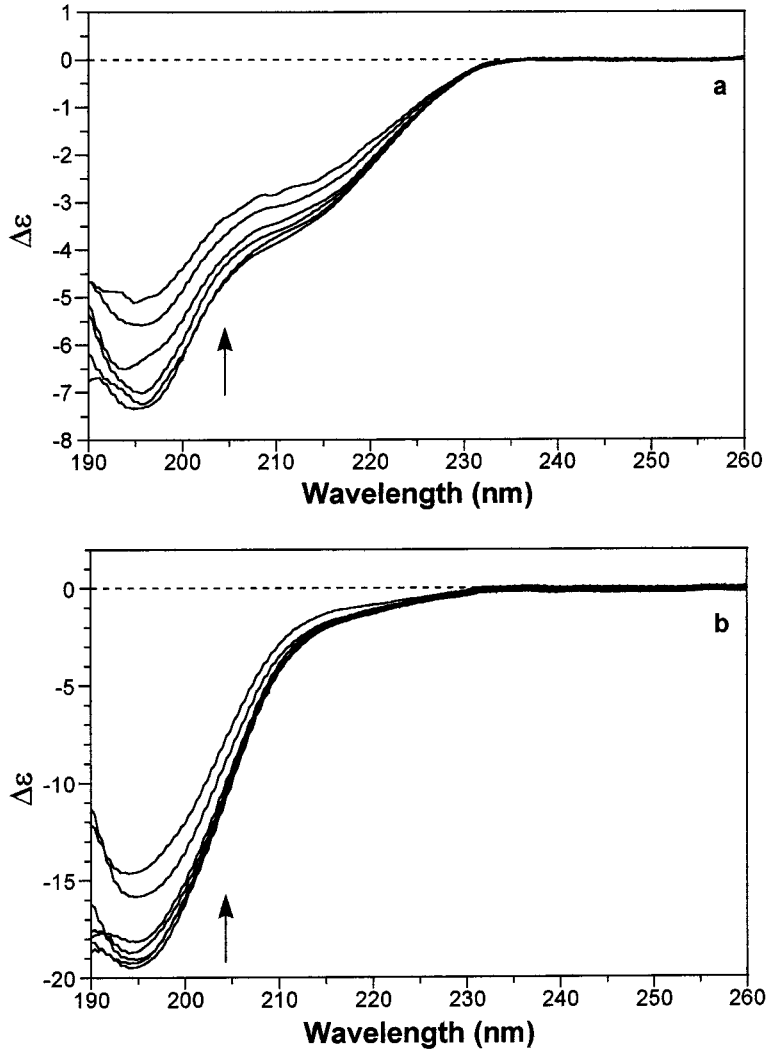


Figure 9. Circular dichroism spectra of **1** in 20% MeOH/H_2O in the presence of an increasing amount of 1-adamantanol (Ada): **a** (1×10^{-4} M, pH 4.1): from bottom to top (arrow) 1:Ada ratios of 1:0, 1:1, 1:2, 1:5, 1:10, 1:20; **b** (6×10^{-5} M, pH 7.1): from bottom to top (arrow) 1:Ada ratios of 1:0, 1:1, 1:2, 1:10, 1:20, 1:50, 1:80

causing an "in and out" movement of the Boc group within the β-CD cavity.

Experimental Section

Materials: β-cyclodextrin hydrate, histamine and Boc-β-alanine were purchased from Sigma. Peptide synthesis grade *N*,*N*-dimethylformamide (DMF, Millipore) was used for the preparation of Boc-carcinine, and anhydrous DMF (Aldrich) was used for the synthesis of **1**. 2-(1-*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and *N*-hydroxybenzotriazole (HOBT) were purchased from Novabiochem. Column chromatography was performed on CM-Sephadex C-25 (40–120 μm, Pharmacia). TLC was conducted on precoated silica gel glass plates (60 F-254, Merck). Melting points were measured with an electrothermal melting-point apparatus and are uncorrected. All pH measurements were made with an Orion Model SA 520 pH meter equipped with a MI410 combined pH electrode. UV/Vis spectra were recorded with a HP 8452A diode array spectrophotometer.

NMR Spectroscopy: Some preliminary ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-250 instrument (250.13 MHz) with 5 mM solutions of **1** in [D_6]DMSO and D_2O.

High field NMR spectra were recorded on a Bruker DRX-600 (600.13 MHz) and a Varian Inova Unityplus-500 spectrometer (499.88 MHz). The experiments were run at 300 K and referenced to the residual HDO peak at $\delta = 4.8$. If not specified otherwise, sample concentration was ca. 5 mM.

1D ^1H NMR spectra were collected during 10 scans using 16 K data points over a spectral width of 4870 Hz. The ^{13}C NMR spectrum was obtained at 62.5 MHz. Homonuclear 2D experiments (DQF-COSY, TOCSY and ROESY) were obtained in the phase sensitive mode with time proportional phase incrementation (TPPI)[33] and were typically acquired with 2 K data points for 512 increments of *t1* (zero filled to 1024 points) recording 16 or 32 transients for each increment. The TOCSY spectrum was acquired using a DIPSI-2[34] with a mixing time of 115 ms and a 8 kHz spin lock field. ROESY spectra were performed at two different mixing times (150 ms and 250 ms) with a 2.5 kHz spin lock field. T-ROESY[35] was obtained at a sample concentration of 1 mM with

a mixing time of 200 ms and 2 K data points for 2 × 512 increments of *t1* (zero filled to 2048 points) recording 24 transients for each increment. Inverse detection proton-carbon correlations experiments, HSQC and HMBC, were carried out with GARP-1 modulated[36] decoupling, acquiring 2 K data points for 256 (HSQC) or 128 (HMBC) increments of *t1* (zero filled to 512 points) with 16 (HSQC) or 140 (HMBC) transients for each increment. Suppression of residual HDO resonance was achieved by low-power presaturation. All NMR spectra were processed with software supplied by the manufacturers.

CD Spectroscopy: Circular Dichroism (c.d.) spectra were obtained at 25°C under a constant flow of nitrogen on a Jasco model J-600 spectropolarimeter which had been calibrated with an aqueous solution of ammonium *d*-camphorsulphate.[37] The measurements were carried out in water and at different pH values using 1 mm or 5 mm pathlength cuvettes. All experiments were performed in the UV region (190–260 nm). The spectra represent the average of 2–10 scans; c.d. intensities are expressed in $\Delta\varepsilon$ (M^{-1} cm^{-1}).

***N-tert*-Butoxycarbonyl-β-alanylhistamine (Boc-Carcinine):** The compound was synthesised from *N-tert*-butoxycarbonyl-β-alanine-(Boc-β-Ala) and histamine (Hm) according to the following procedure: histamine (0.78 g, 7.0 mmol) was added to a solution of *N-tert*-butoxycarbonyl-β-alanine (1.47 g, 7.8 mmol) in the presence of HOBT (1.19 g, 7.8 mmol) and TBTU (2.50 g, 7.8 mmol) in DMF (10 mL). The reaction mixture was stirred at room temperature for two hours under nitrogen. Progress of the reaction was followed by TLC (eluent: $CHCl_3/CH_3OH/H_2O$ 65:25:4). The solution was evaporated to dryness in vacuo, dissolved in a minimum amount of water and applied to a column (3 × 90 cm) of CM-Sephadex C-25 (NH_4^+ form). The column was eluted initially with water (1000 mL), and then with a linear gradient of aqueous NH_4HCO_3 (0–0.1 M, 3000 mL). The product was detected with the Pauli spray reagent.[30] The fractions containing the pure product were combined, concentrated to dryness under vacuum at 40 °C, repeatedly dissolved in water and dried to let ammonium hydrogen carbonate decompose. The product obtained (1.5 g, 68% yield) was a white solid: M.p. 147–148 °C. – FAB-MS: *m/z* = 283 $[M + H]^+$. – $C_{13}H_{22}N_4O_3$: calcd. C 55.29, H 7.86, N 19.85; found C 54.98, H 7.79, N 19.93. – ^1H NMR (250 MHz, D_2O): δ = 1.38 (s, 9 H, α-CH_3), 2.33 [t, ^3J(H,H) = 6.40 Hz, 2 H, ε-CH_2], 2.79 [t, ^3J(H,H) = 6.64 Hz, 2 H, θ-CH_2], 3.26 [t, ^3J(H,H) = 6.40 Hz, 2 H, δ-CH_2], 3.42 [t, ^3J(H,H) = 6.64 Hz, 2 H, η-CH_2], 6.98 (s, 1 H, κ-CH), 7.88 (s, 1 H, λ-CH). – ^{13}C (62.89 MHz, D_2O): δ = 28.0 (θ-CH_2), 30.2 (α-CH_3), 38.7 (ε-CH_2), 39.3 (δ-CH_2), 41.3 (η-CH_2), 83.6 (β-C_{quat}), 119.4 (κ-CH), 136.2 (ι-C_{quat}), 137.8 (λ-CH), 160.5 (γ-CO), 176.7 (ζ-CO).

6-{4-[*N-tert*-Butoxycarbonyl-*N*-(*N'*-ethyl)propanamide]imidazolyl}-6-deoxycyclomaltoheptaose (1): This compound was synthesised by the reaction of 6-deoxy-6-iodocyclomaltoheptaose (CDI)[31] (300 mg, 0.24 mmol) with Carc-Boc (350 mg, 1.2 mmol) in anhydrous DMF (12 mL) at 90 °C under nitrogen. The progress of the reaction was followed by TLC (eluent: *n*PrOH/H_2O/EtOAc/NH_3, 5:3:2:1). After 72 hours, DMF was evaporated, the residue was dissolved in water and applied to a CM-Sephadex C-25 (NH_4^+ form) column (2 × 60 cm). The column was eluted with water (600 mL). Fractions were assayed by TLC (same eluent as above). The product was detected with anisaldehyde reagent.[32] The fractions containing the product (r_f = 0.24) were combined and concentrated to dryness under vacuum at 40 °C. The final product was further crystallised from water to give 0.19 g (0.14 mmol, yield 58%, based on CDI) of colourless needles. M.p. 285 °C (dec.). – FAB-MS spectrometry: *m/z* = 1400 $[M + H]^+$. – $C_{55}H_{90}N_4O_{37}\cdot 9H_2O$: calcd. C 42.29, H 6.97, N 3.59; found C 42.05, H 7.00, N 3.63. – ^1H NMR (250 MHz, [D_6]DMSO, the chemical shift values refer to the residual DMSO peak at δ = 2.5) β-cyclodextrin: δ = 5.90–5.48 (m, 14 H, 2-OH, 3-OH), 4.82 [m, 8 H, H(1), 6-OH], 4.68 (t, 1 H, 6-OH), 4.61 (t, 1 H, 6-OH), 4.49 (m, 3 H, 6-OH), 4.31 [m, 2 H, H(6)1, 6-OH], 4.00 [m, 1 H, H(6′)1], 3.89 [m, 1 H, H(5)1], 3.60–3.24 [m, 36 H, H(6)2–6, H(3), H(5)2–7, H(4)2–7, H(2)], 3.12 [m, 6 H, H(6)7, H(6′)7, H(4)1]. Substituent: δ = 7.89 (t, 1 H, histamine-NH), 7.54 (s, 1 H, λ-CH), 6.84 (s, 1 H, κ-CH), 6.69 (t, 1 H, β-alanine-NH), 3.22 (m, 2 H, η-CH_2), 3.09 (m, 2 H, δ-CH_2), 2.50 (m, 2 H, θ-CH_2), 2.17 (m, 2 H, ε-CH_2), 1.37 (s, 9 H, α-CH_3).

Crystal Structure: Transparent crystals of β-CD-Boc-carcinine (**1**) with a regular parallelepiped structure suitable for X-ray diffraction were obtained at room temperature by slow evaporation of an aqueous solution. A crystal the size of 0.3 × 0.5 × 0.5 mm was used for X-ray study. The crystal system and space group were determined by preliminary oscillation and Weissenberg photographs. Cell dimensions were determined by a least-squares fit of the experimentally determined positions of 25 high angle reflections (2θ range 45–50°). The data was collected on a CAD4 turbo Enraf–Nonius diffractometer with graphite-monochromatised CuKα radiation (λ = 1.54178 Å) and an ω-2θ scan mode up to 140 in 2θ. The intensities of three reflections, used as standards, were measured every 60 min of X-ray exposure time and showed an average random fluctuation of ca. 3%. Detailed crystallographic data are presented in Table 7. A total of 7245 independent reflections were measured and corrected for Lorentz and polarisation factors. Of these 6779 reflections have a net intensity $I > 2.0\sigma(I)$. No absorption correction was applied. The space group symmetry ($P2_1$) and unit cell dimensions suggested the presence of one β-CD molecule and several water molecules in the crystal asymmetric unit. Many attempts to solve the structure by the straightforward application of direct methods failed. The structure was solved by the Patterson search program PATSEE[38] by using the atomic coordinates of three glycosidic units of 6-deoxy-6-{4-[*N-tert*-butoxycarbonyl-2-aminoethyl]-imidazolyl}-cyclomaltoheptaose[8b] for the rotation search. The best result was used to assign the phases to 500 E values and to partially expand the set by SIR-97.[39] The E-map revealed the position of all atoms of the

Table 7. Crystal data and structure refinement parameters

Empirical formula	$C_{55}H_{90}N_4O_{37}\cdot 9H_2O$
Formula weight	1560.6
Temperature	293(2) K
Wavelength	1.54178 A
Crystal system, space group	Monoclinic, $P2_{sl}$
Unit cell dimensions	*a* = 15.116(2) Å
	b = 16.016(4) Å
	c = 15.274(4) A
	β = 93.03(2)°
Volume	3693(1) A^3
Z, Calculated density	2, 1.39 Mg/m^3
Absorption coefficient	0.917 mm^{-1}
Theta range for data collection	2.90 to 69.92°
Index ranges	–18 *h* 18
	–19 *k* 18
	0 *l* 18
Reflections collected/unique	7245/7245
Completeness to 2θ = 69.92	99.7
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	7245/1/937
Goodness-of-fit on F^2	1.019
Final R indices [$I > 2\sigma(I)$]	*R*1 = 0.0625, *wR*2 = 0.1829
R indices (all data)	*R*1 = 0.0663, *wR*2 = 0.1932
Largest diff. peak and hole	0.660 and –0.508 e.A^{-3}

molecule and some water molecules. Subsequent difference electron density maps revealed the positions of the remaining water molecules. The structure was refined using the full-matrix least-squares program SHELXL-93.[40] The refinement was carried out on Fo^2 values using 937 parameters including atomic coordinates and anisotropic thermal factors for N, C and O atoms, and isotropic thermal factors for the Ow6, Ow7, Ow8, and Ow9 water molecules. All hydrogen atoms were included in their stereochemically expected positions with thermal factors equal to the equivalent U of the carrier atom (C–H distance 0.96 Å), except those of the water molecules. Subsequent difference Fourier analysis revealed 9 cocrystallised water molecules, and the statistical disorder of the O(6)3 atom over two sites with site occupancy factors of 0.5. Scattering factors were taken from the International Tables for X-ray Crystallography.[41] All measurements and calculations were carried out at the Biocrystallography Research Centre of the CNR at the University of Naples. The structure was refined to the final indices $R1 = 0.0625$ and $wR2 = 0.182$. In Scheme 1 the molecule is shown with atom labels for the first glycosidic residue and the Boc-carcinine moiety.

Crystallographic data (excluding structure factors) for the structure(s) included in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-134049. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

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