A Modified Cyclodextrin with a Fully Encapsulated Dansyl Group: Self-Inclusion in the Solid State and in Solution

Roberto Corradini,* Arnaldo Dossena, Rosangela Marchelli, Anna Panagia, Giorgio Sartor, Michele Saviano, Angela Lombardi, and Vincenzo Pavone*

Abstract: A monofunctionalized β-cyclodextrin containing a dansyl moiety, 6-deoxy-6-*N*-(*N'*-(5-dimethylamino-1-naphthalenesulfonyl)diaminoethane)-β-cyclodextrin (CD-en-DNS, **2**), was synthesized and its crystal structure determined. It was shown that the dansyl group is fully encapsulated within the cyclodextrin cavity, with the dimethylamino and sulfonyl groups emerging from opposite sides. The shape of the cavity is considerably flattened, since O(4)–O(4) distances parallel to the naphthalene ring were found to be longer than the others. The conformation of the diaminoethane linker was found to be determined by the inclusion of the dansyl group and by a hydrogen bond between the sulfonamide NH and one of the O(6)–H groups on the cyclodextrin rim. The self-inclusion features of the aromatic moiety were found to be consistent with the solution data: ^{1}H NMR ROESY spectra suggested that the orientation of the dansyl moiety observed in the solid state was retained in aqueous solution; the circular dichroism spectrum was consistent with an axial complexation model. Fluorescence spectra showed that the inclusion of the dansyl group in the cyclodextrin cavity considerably increases the quantum yield; time-resolved fluorescence experiments showed the presence of a long-lifetime component (16.1 ns), which was attributed to the included fluorophore. The ability of **2** to act as a fluorescence sensor was evaluated by the addition of several guests of different shape: fluorescence intensity was lowered, especially upon addition of adamantanecarboxylic acid. All the data obtained were consistent with the model of the in–out movement of the dansyl group from the self-included conformation observed in the solid state to a position more exposed to the bulk solvent. Copper(II) was shown to enhance the difference in the fluorescence of **2** in the presence of guests by additional static quenching.

Keywords
crystal structure · cyclodextrins · dansyl derivatives · fluorescent sensors · self-inclusion

Introduction

α-, β-, and γ-Cyclodextrins (cyclic oligosaccharides composed of six, seven, and eight D-(+)-glucopyranose units, respectively)[1] in aqueous solution can include a variety of organic compounds within their hydrophobic cavities without formation of covalent bonds.[2] Thus, they have been extensively used for molecular recognition of neutral molecules, being able to discriminate between guests of different shapes and dimensions, and as building blocks for supramolecular structures.[3] In particular, β-cyclodextrins show a strong preference for compounds containing polycyclic aromatic groups, such as naphthalene[4] or pyrene, and tricyclic aliphatic moieties, such as adamantanecarboxylic acid (ACA).[5] It has yet to be resolved whether the inclusion of lipophilic molecules within the cavity should be attributed to an entropic "hydrophobic" effect, or to dispersion forces giving rise to attractive interactions.[6]

A large number of cyclodextrins bearing substituents with specific functional groups have been synthesized in order to achieve a more efficient and/or selective binding of molecules with complementary functionalities.[7] Recently, great attention has been paid to the use of cyclodextrins in luminescence studies[8] and in nonlinear optics.[9] Unmodified cyclodextrins have been used as fluorescence-enhancing agents for analytical purposes,[10] and the competitive binding of a fluorophore is a technique widely used for the determination of the stability constants of cyclodextrins host–guest inclusion complexes.[11]

In recent years, Ueno and his collaborators have utilized these properties for obtaining optical sensors based on molecular recognition,[12] by covalently linking a chromophore or fluorophore to cyclodextrins. Cyclodextrins linked to ferrocene,[13] anthracene,[14] pyrene,[15] naphthalene,[16] fluorescein,[17] *p*-dimethylaminobenzoyl (DMAB),[18] or dansyl[19, 20] units showed guest-induced variations in circular dichroic, absorption, and fluorescence spectra, and exhibited remarkable molecular selectivity toward steroidal systems.

Bright and co-workers have investigated the time-resolved fluorescence of one of these molecules, a dansylglycine-modified β-cyclodextrin,[21] and shown it to be an optical sensor of re-

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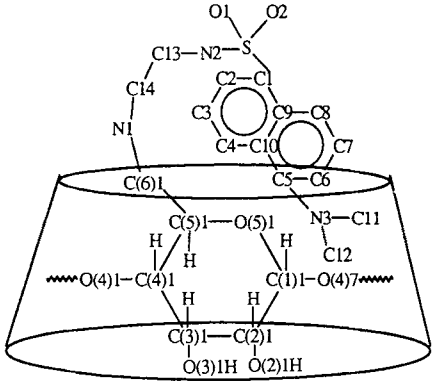
  0947-6539/96/0204-0373 $ 15.00+.25/0

markable stability once trapped in a sol-gel matrix.[22] In this system, the self-inclusion of the fluorophore within the cyclodextrin cavity was postulated to be an essential feature for the sensing properties, although complete self-inclusion was not observed in the available X-ray crystal structures of monofunctionalized β-cyclodextrins.

Inclusion of dansyl derivatives within unmodified cyclodextrins has also been proposed to be involved in the mechanism of chiral discrimination in high-performance liquid chromatography (HPLC),[23] thin-layer chromatography (TLC),[24] and capillary-zone electrophoresis (CZE).[25] The use of β-cyclodextrin for the enantioselective crystallization of *N*-dansyl α-amino acids has also been reported.[26] The observed enantioselectivity in these systems is generally attributed to the presence of some additional interaction (e.g., hydrogen bonding) between the included guest and the cyclodextrin rim. However, to our knowledge, very few structural data are available to support the numerous pictorial molecular models proposed.

We report here the crystal structure of a β-cyclodextrin monofunctionalized at C(6) with *N*-dansylethylenediamine (en-DNS, **1**), 6-deoxy-6-*N*-(*N'*-(5-dimethylamino-1-naphthalenesulfonyl)-diaminoethane)-β-cyclodextrin (CD-en-DNS, **2**; Scheme 1), which for the first time provides direct evidence with full structural details for the inclusion of the dansyl group within the cyclodextrin cavity.



Scheme 1. General formula and numbering of **2**. The cyclodextrin protons are indicated as C(*m*)*n*, where *m* is the glucose proton number and *n* is the number of the glucose unit.

We carried out a spectroscopic investigation in aqueous solution, in order to determine whether or not the structural features observed in the solid state were retained. The preferred conformations of **2** in aqueous solution were studied by CD, NMR, and fluorescence spectroscopy, and compared with the crystal structure. The ability of **2** to act as a fluorescent sensor for neutral molecules, analogously to other dansyl-modified cyclodextrins, was also investigated. In fact, CD-en-DNS (**2**) should undergo a pronounced decrease in the fluorescence intensity upon expulsion of the DNS group from the cavity, mainly due to the dynamic (i.e., collisional) quenching by solvent molecules.

We investigated the effect of copper(II) ions on the sensing properties of **2**, to establish whether additional (static) quenching effects occur on complexation,[27] as recently demonstrated by some of us for the preferentially inclusion of D-tryptophan by the copper(II) complex of a histamine-monofunctionalized β-cyclodextrin.[28],[29]

Results and Discussion

The synthesis of CD-en-DNS (**2**) was carried out (with a modification of a literature procedure[30]) by reaction of β-cyclodextrin with tosyl chloride in pyridine, substitution of the resulting mono-6-*O*-tosylate with iodide, and reaction of the 6-deoxy-6-iodo-β-cyclodextrin with the amine en-DNS (**1**) (obtained by reaction of excess ethylenediamine with dansyl chloride). A good yield (50%) was obtained for the last step of the synthesis, compared to that previously reported for a dansylglycine-β-cyclodextrin.[16]

Crystal structure of 2: The molecular structure of **2** is shown as stereoviews in Figure 1. The overall shape of the molecule corresponds to a distorted truncated cone. The en-DNS moiety forms a folded structure directing the terminal DNS group inside the cavity; the latter is fully encapsulated. Although many inclusion complexes of α-, β-, or γ-cyclodextrins have been investigated, very few crystal structures of monofunctionalized cyclodextrins have been reported, and none of these provided evidence of complete self-inclusion of the substituent within the cyclodextrin cavity. Inclusion of the side arm within the cavity of an adjacent cyclodextrin has been observed in the case of 6-deoxy-6-*tert*-butylthio-,[31] 6-deoxy-6-phenylthio-,[32] and 6-deoxy-6-phenylsulfinyl-β-cyclodextrin.[32] Self-inclusion of the leucine side chain has been observed in the case of 6-deoxy-6-cyclo-(L-histidyl-L-leucyl)-β-cyclodextrin.[33] Therefore, the crystal structure of **2** provides the first example of a fully self-included β-cyclodextrin derivative.

The inclusion of the dansyl group causes a remarkable distortion of the heptagonal symmetry of the β-cyclodextrin moiety, so that it is wider along the imaginary axis connecting the centers of the G 3 and G 7 glucose units. The aromatic ring is nearly perpendicular to the mean square plane formed by the O(4) atoms (the dihedral angle is 79.6°); the longest side of the naphthalene ring points toward the glucose rings G 3 and G 7. The sulfonyl and dimethylamino groups point outward from the

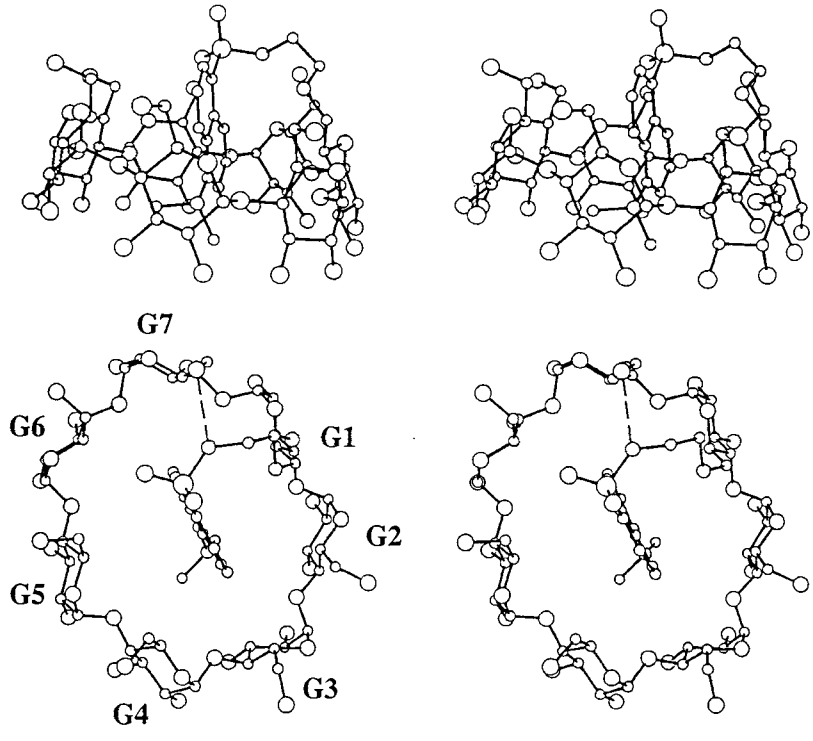


Fig. 1. Stereoview of the crystal structure of CD-en-DNS (**2**). G1–7 refer to the glucose units.

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β-cyclodextrin truncated cone on opposite sides; the former is closer to the plane formed by the O(6) atoms.

All the glucose residues have a $^4C_1$ chair conformation. All the secondary hydroxyl groups (O(2)–H and O(3)–H) form intramolecular hydrogen bonds with neighboring glucose residues (see Table 1), thus maintaining the round shape of the β-cyclodextrin ring. The O(2)$n\cdots$O(3)$n-1$ distances are in the range 2.73–2.88 Å, in agreement with other known β-cyclodextrin crystal structures.[34]

Table 1. Inter- and intramolecular H bonds in **2**: distances d between O(2)n and O(3)$n-1$, between selected atoms in the β-CD moieties and water oxygen atoms (O_w), and between water molecules.

		d (Å)	Symmetry operation
O(2)1	O(3)7	2.88	
O(2)2	O(3)1	2.79	
O(2)3	O(3)2	2.87	
O(2)4	O(3)3	2.77	
O(2)5	O(3)4	2.83	
O(2)6	O(3)5	2.78	
O(2)7	O(3)6	2.73	
O(6)6	O_w1	2.78	$x-1/2, 3/2-y, 1-z$
O(2)7	O_w2	2.70	$x-1/2, 3/2-y, 1-z$
O(2)5	O_w3	2.93	x, y, z
O(3)5	O_w3	3.14	x, y, z
O(6)7	O_w3	3.08	$-x, y-1/2, 3/2-z$
N1	O_w4	2.79	x, y, z
O(3)6	O_w4	2.76	$-x, y+1/2, 3/2-z$
O(6)7	O_w5	2.83	$x-1/2, 3/2-y, 1-z$
O(3)5	O_w6	2.79	x, y, z
O(2)5	O_w7	2.65	x, y, z
O(2)1	O_w8	2.72	$-x, y+1/2, 3/2-z$
O(6)4	O_w8	3.16	x, y, z
O(6)5	O_w8	3.28	x, y, z
O(6)4	O_w9	3.25	$1/2-x, 1-y, 1/2+z$
O(2)2	O_w10	2.83	$-x, y+1/2, 3/2-z$
O(6)3	O_w10	2.73	$x, y, z+1$
O(2)6	O_w11	2.96	x, y, z
O(6)4	O_w12	2.66	$1/2-x, 1-y, 1/2+z$
O(3)7	O_w12	2.75	$x-1/2, 3/2-y, 1-z$
O(6)5	O_w14	2.66	x, y, z
O_w1	O_w5	2.77	x, y, z
O_w2	O_w9	2.69	x, y, z
O_w3	O_w11	2.74	$x-1/2, 3/2-y, 2-z$
O_w4	O_w7	2.82	$1/2-x, 2-y, 1/2+z$
O_w5	O_w6	2.78	x, y, z
O_w5	O_w16	3.11	x, y, z
O_w6	O_w11	2.83	x, y, z
O_w6	O_w16	3.17	x, y, z
O_w8	O_w9	2.92	x, y, z
O_w8	O_w10	3.26	$1/2-x, 1-y, 1/2+z$
O_w8	O_w15	3.30	x, y, z
O_w9	O_w10	3.08	$1/2-x, 1-y, 1/2+z$
O_w9	O_w12	2.84	x, y, z
O_w10	O_w13	2.59	x, y, z
O_w11	O_w13	2.81	x, y, z
O_w12	O_w14	2.63	x, y, z
O_w13	O_w14	2.51	x, y, z
O_w13	O_w16	3.48	x, y, z
O_w14	O_w15	3.02	x, y, z
O_w14	O_w16	2.61	x, y, z
O_w15	O_w16	2.75	x, y, z

Table 2 reports in detail the β-cyclodextrin ring parameters. The seven glycosidic O(4) atoms are coplanar within 0.26 Å, forming a distorted heptagon with sides of 4.10–4.61 Å in length and radii of 4.54–5.45 Å. It is worth noting that the sides of the heptagon facing the naphthalene plane are significantly longer than the other sides (the O(4)7–O(4)1 and O(4)4–O(4)5 distances are 4.6 Å); in particular, they are longer than the mutually opposing sides O(4)6–O(4)7 (4.1 Å) and O(4)2–O(4)3 (4.2 Å). The glucose residues are inclined towards the perpendicular axis of the plane formed by the O(4) atoms, making the upper rim with the primary hydroxyl groups smaller. The tilt angles[35] of the glucose residues are in the 4.37–23.04° range, with the larger values for the glucose units 1 and 4. The [O(4)$n-1$]–[O(4)n]–[O(4)$n+1$] angles are in the 118.4–137.8° range, and are wider around the O(4)1, O(4)4, and O(4)5 atoms. All the C(6)–O(6) groups are pointing outward from the torus, and are involved in hydrogen bonds with the surrounding cocrystallized water molecules, except that of glucose unit 7, which is rotated inward to allow the formation of a hydrogen bond with the sulfonamide N2 atom of the en-DNS group (the N2–O(6)7 distance is 2.97 Å).

Table 2. Geometrical data concerning to the β-cyclodextrin rings in **2** (see Fig. 1 for numbering).

	r (Å) [a]	d (Å) [b]	θ (°) [c]	ϕ_{tilt} (°) [d]	P (Å) [e]
G1	4.54	4.40	137.8	23.04	0.102
G2	5.22	4.22	122.8	4.99	0.208
G3	5.30	4.25	123.6	6.12	−0.265
G4	4.78	4.60	132.6	19.84	−0.042
G5	4.76	4.35	134.3	6.93	0.259
G6	5.45	4.10	118.4	4.37	−0.068
G7	5.03	4.61	128.5	8.36	−0.194
average	5.01	4.36	128.3	10.52	0.163
mean values [f]	5.1(1)	4.4(1)	118(1)	12(10)	0.178

[a] The radius r is measured from the centre of gravity of the seven O(4) atoms to each O(4) atom. [b] Defined as the O(4)$n\cdots$O(4)$n+1$ distance. [c] Defined as the [O(4)$n-1$]–[O(4)n]–[O(4)$n+1$] angle. [d] The tilt angle ϕ_{tilt} is defined as the angle between the plane formed by the O(4) atoms and the plane formed by O(4)$n+1$, C(1)n, C(4)n, and O(4)n atoms of each glucose residue. [e] The planarity P is defined as the O(4)n displacement from the mean plane formed by the O(4) atoms. [f] From ref. [34]; standard deviations are reported in parentheses.

The encapsulation of the DNS group within the β-cyclodextrin cavity is achieved by a folding of the ethylendiamine bridge with the C(6)1–N1 bond pointing inward. The O(5)1-C(5)1-C(6)1-N1 torsion angle is *gauche*(+), while, along the chain from C(6)1 to N2, a *gauche*(−)–*trans*–*gauche*(−)–*skew* succession of torsion angles ensures the appropriate positioning of the DNS center near the center of the β-cyclodextrin cavity. This folded conformation is stabilized by a hydrogen bond between the unusually inward oriented O(6)7 primary hydroxyl group and the N2 atom, as mentioned above. Furthermore, the N1 atom is involved in hydrogen bonding with a water molecule (the O_w4–N1 distance is 2.8 Å). This water molecule is also hydrogen bonded with O_w7 and O(3)6, belonging to a symmetry related molecule. In turn, O_w7 also participates in a hydrogen bond with O(2)5, in a symmetry related molecule. Thus O_w4 and O_w7 act as a bridge and stabilize columns of molecules along the b direction. Neither nitrogen nor oxygen atoms of the sulfonamide group form hydrogen bonds with solvent molecules.

The aromatic part of DNS group is fully encapsulated within the cavity. Atoms C11, C12, and N3 are below the O(4) plane (on the side of O(2)n and O(3)n secondary hydroxyl groups). Van der Waals contacts between the DNS group and the cyclodextrin moiety are reported in Table 3, indicating that the DNS atoms C3, C4, C5, C6, C11, and C12 are the main atoms involved in the inclusion phenomenon.

The crystal packing along the b axis is reported in Figure 2. Columns oriented along the b axis are formed by macrocycles related by a 2_1 screw axis and are inclined with respect to this axis by about 28°. In the crystal packing, no direct link between primary and secondary hydroxyl groups of symmetry related units are present. Sixteen equivalents of cocrystallized water

Table 3. Main van der Waals contacts (d<4.5 Å) between the DNS moiety and the β-cyclodextrin in **2**.

DNS	β-CD	d (Å)	DNS	β-CD	d (Å)
N2	C(5)7	4.29	C4	C(6)2	4.39
N2	C(6)7	3.82	C4	C(4)3	4.45
S	C(6)6	4.49	C4	O(4)3	3.90
S	O(6)7	4.30	C4	C(5)3	4.47
O1	C(6)5	3.56	C4	C(6)4	4.21
O1	C(5)6	3.74	C5	O(4)1	4.30
O1	O(5)6	4.41	C5	C(5)1	4.41
O1	C(6)6	3.30	C6	O(4)1	4.50
O1	O(6)7	4.24	C6	C(5)1	4.23
C1	C(6)5	4.40	C11	C(3)2	3.83
C2	C(6)4	4.08	C11	O(3)2	4.29
C3	C(6)2	4.45	C11	O(4)2	4.22
C3	O(4)3	4.33	C12	C(3)3	3.91
C3	C(5)3	4.11	C12	O(3)3	4.14
C3	C(6)3	4.16	C12	C(4)3	4.35
C3	C(5)4	3.84	C12	O(4)3	3.63
C3	O(5)4	4.38	C12	C(3)4	3.77
C3	C(6)4	3.79	C12	C(4)4	4.09
C4	O(4)2	4.23	C12	O(4)4	3.66
C4	C(5)2	4.24	C12	C(5)4	4.16

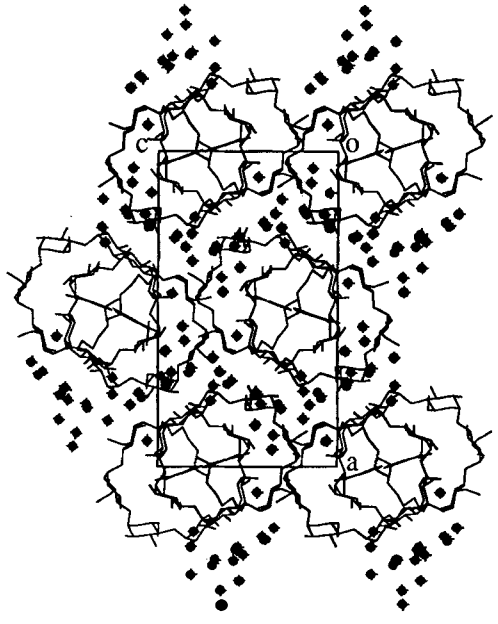

Fig. 2. Crystal packing viewed along the b axis. Water molecules (•) are also included.

molecules are involved in a network of hydrogen bonds in the crystal. The water molecules also fill the lattice space. Table 1 reports the hydrogen-bond distances.

Spectroscopic evidence for self-inclusion in aqueous solution: The conformation of **2** in aqueous solution was investigated by NMR, CD, and fluorescence spectroscopy, in order to establish whether the self-inclusion features of the dansyl group within the cavity were retained.

The 400 MHz ^{1}H NMR spectrum in deuterium oxide exhibits severe overlapping of peaks in the aliphatic region. Seven separate resonances are observed for the anomeric H(1) protons; in β-cyclodextrin only one degenerate H(1) resonance is observed. In the COSY and TOCSY spectra seven cross-peaks, corresponding to the correlation between H(1) and H(2) protons in a monosubstituted cyclodextrin, were observed, allowing the assignment of all the H(2) protons (data not shown). The aromatic protons were assigned on the basis of the COSY and ROESY[36] spectra. In particular, two series of signals corresponding to atoms connected through bonds were identified in the COSY spectrum and assigned to the naphthalene aromatic rings; the connectivity through space with the protons of the dimethylamino group allowed the identification of the protons H4 and H6 of the naphthalene unit and of the other aromatic signals. The combined use of TOCSY and ROESY experiments allowed us to assign all the protons of the ethylenediamino linker and most of the signals of the cyclodextrin protons. In particular, according to a strategy developed for cyclodextrins and oligosaccharides, the signals of each glucose unit could be identified from TOCSY cross-peaks; several spectra were recorded with increasing spin-lock times (from 30 to 90 ms), and the H(1)–H(2), H(1)–H(3), H(1)–H(4), and H(1)–H(5) connectivities were thus progressively detected; H(6) protons were assigned from cross-peaks with the H(5) and H(4) protons. The sequence by which the glucose units are connected could be obtained from the ^{1}H–^{1}H ROESY cross-peaks between the H(1)i anomeric proton of one glucose unit and H(4)$i-1$ of the preceding glucose residue.[37] The peak assignment of the cyclodextrin protons obtained by this approach are reported in Table 4.

Table 4. ^{1}H NMR peak assignments based on TOCSY and ROESY spectra (400 MHz, D_2O, 310 K).

	H(1)	H(2)	H(3)	H(4)	H(5)	H(6)
G1	5.00	3.64	3.65	3.17	2.69	2.42 2.10
G2	4.99	3.73	4.05	3.72	3.36	3.63 3.83
G3	5.32	3.91	4.47	3.93	4.47	4.16 4.30
G4	5.19	3.71	3.86	3.58	3.49	3.70 3.25
G5	4.94	3.62	3.55	3.50	2.58	3.20 3.50
G6	5.18	3.82	4.17	3.75	4.17	4.20 4.30
G7	5.34	3.85	4.32	3.71	4.32	3.83 4.05

The proton chemical shifts are spread over a wide range, owing to the magnetic anisotropic effect of the naphthalene ring: the shielding and deshielding effects observed suggest a disposition of the naphthalene ring similar to that observed in the solid state. In fact, the protons of the glucose units G3, G6, and G7, which face the edge of the aromatic group in the crystal structure, generally have high chemical shifts, while those of G1 and G5, which are above and below the plane of the aromatic ring, have the lowest chemical shifts for all protons. The H(5) protons are particularly sensitive to anisotropic effects, since their chemical shifts vary from 2.58 for H(5)5 and 2.69 for H(5)1 to 4.47 for H(5)3; a similar, but less pronounced, effect was found for H(3) protons. This is also in agreement with the solid-state structure, since both H(5) and H(3) are located inside the cavity and are close to the aromatic moiety, with the former being located close to the center of the naphthalene ring. A portion of the ROESY spectrum, containing cross-peaks between the aromatic protons and those of the cyclodextrin cavity, is reported in Figure 3.

In Table 5 the observed connectivities are compared with the distances observed in the solid state. Figure 4 shows the corresponding protons in the crystal structure. The aromatic H2 and H6 protons show no NOE with the cyclodextrin protons, in accordance with the solid-state structure, in which the two protons are located near the cyclodextrin axis. The other connectiv-

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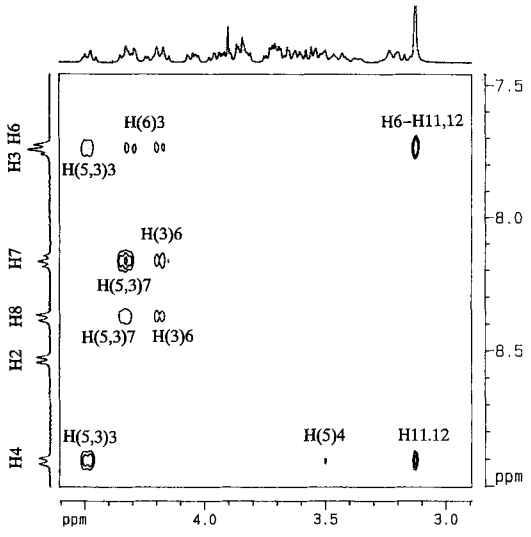

Fig. 3. Contour plot of a portion of the 400 MHz ROESY spectrum of **2** in D_2O at 310 K.

Table 5. ROESY cross-peaks and crystal-structure nearest-neighbor distances (d) between the naphthalene protons and protons within the cyclodextrin cavity (400 MHz, D_2O, 310 K).

DNS H	β-CD H	d (Å)	Intensity
H2	H(6)4	3.52	no signal
H3	H(6)3	2.77	+
	H(5)3	2.83	+
H4	H(5)3	2.21	+ +
	H(5)4	2.84	+
H6	H(3)7	3.7	no signal
H7	H(3)7	2.35	+ + + [a]
	H(5)7	2.40	+ + + [a]
	H(3)6	2.80	+ [b]
	H(5)6	2.90	+ [b]
H8	H(5)6	2.36	+
	H(5)7	2.40	+

[a] The signals of H(3)7 and H(5)7 overlap. [b] The signals of H(3)6 and H(5)6 overlap.

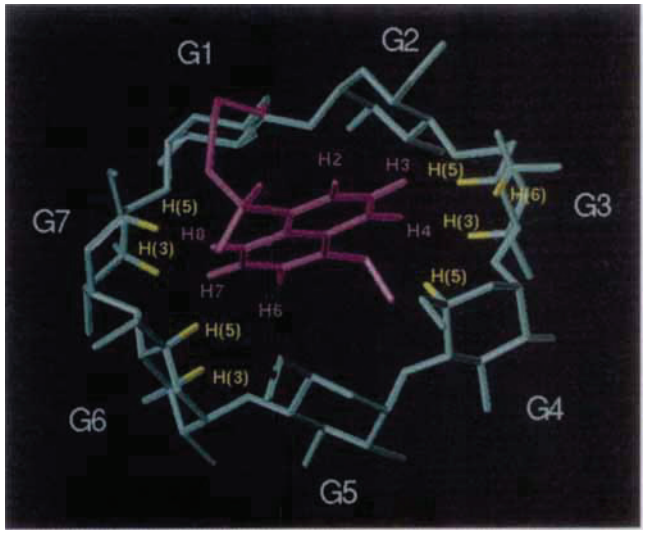

Fig. 4. Crystal Structure of **2**. Magenta: dansylethylenediamine group; yellow: protons of the cyclodextrin cavity showing NOE with those of the dansyl moiety in solution.

ities are also in agreement with this structure, since protons H3 and H4 are close to the glucose units G3 and G4, and protons H7 and H8 are near the glucose units G6 and G7.

Further insight into the self-inclusion was obtained from the UV and CD spectra, by taking advantage of the fact that an achiral guest molecule included in the chiral cyclodextrin cavity may exhibit an induced circular dichroism (ICD) in its absorption regions. In an α-amino-substituted naphthalene such as the DNS group, the ^1L_a and ^1L_b states are of similar energy, owing to the red-shift of the ^1L_a band (depending on the polarity of the solvent) and are probably mixed for 1,5-DNS amides and related compounds in aqueous solution.[38]

For simple chromophores, such as substituted benzene and naphthalene rings, it has been possible to correlate, on a theoretical basis, the sign of the observed ICD with the orientation of the dipole transition moment of a given absorption band relative to the β-cyclodextrin sevenfold axis.[39] Substituted naphthalenes included in cyclodextrins show CD bands for ^1L_a and ^1L_b of the same sign and opposite to that of the ^1B_b transition. The following correlations have been derived: with axial complexation (i.e., with the long axis of naphthalene parallel to the β-cyclodextrin sevenfold axis) ^1B_b has a positive and ^1L_a a negative sign; opposite signs are expected for equatorial complexation.[4b, 40]

UV and CD absorptions of **2** (Fig. 5) are consistent with the axial inclusion mode of naphthalene: the band at 350 nm can be attributed to the overlapping ^1L_a and ^1L_b transitions, and the band at 260 nm to the ^1B_b transition (long axis polarized). This observation is in agreement with the solid-state structure, in which the naphthalene long axis is inclined slightly away from the β-cyclodextrin approximate sevenfold axis.

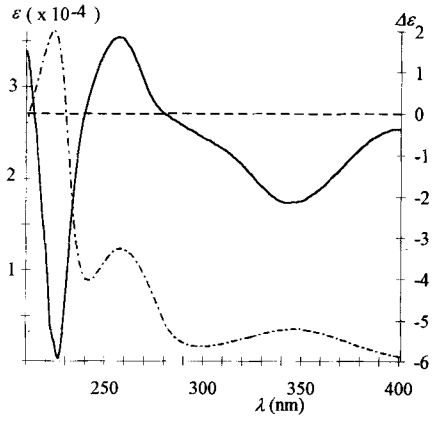

Fig. 5. Absorption (· – ·) and circular dichroism (—) spectra of **2** in H_2O (tetraborate buffer, pH = 8.0).

Fluorescence measurements were carried out on both the dansylated cyclodextrin **2** and on the parent en-DNS (**1**) in aqueous solution (Fig. 6). The intensity of the spectrum of **2** was found to be much greater than that of the reference amine. The relative quantum yield was found to be 28 times higher for **2** than for **1**. The effect of cyclodextrins on fluorescence intensity is generally attributed to the protection of the fluorophore included in the cavity from quenching by the solvent molecules or by other species present in solution. Enantioselective fluorescence responses have also been observed for binaphthyl derivatives as a consequence of preferential inclusion.[41]

The emission wavelength of dansyl derivatives has been reported to be dependent upon the solvent polarity: shorter wavelengths are observed at low Dimroth's E_T values, owing to the

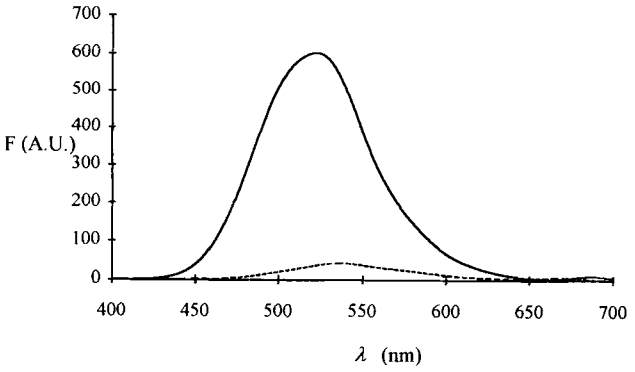

Fig. 6. Fluorescence emission spectra of **1** (- -) and of **2** (—) in H_2O (tetraborate buffer, pH = 8.0).

polarity of the emitting ^1L_a state, which is thought to have charge-transfer character.[38] In the present case, a blue-shift was observed in the emission maximum of **2** (522 nm) as compared to that of **1** (538 nm); this suggests that the fluorophore of **2** experiences a less polar environment.

Time-resolved fluorescence experiments, carried out using the single-photon counting technique,[42] showed the presence of a component with a long lifetime for **2** (16.1 ns), which was absent in the case of **1** (Table 6). The higher fluorescence intensity

Table 6. Fluorescence lifetime (τ) and molar fractions (α) for dansylated amines and cyclodextrins in aqueous solution (pH = 8.0) [a].

	Guest	τ_1	α_1	τ_2	α_2	τ_3	α_3
1	–	3.3	0.40	2.2	0.40	1	0.20
2	–	16.1	0.90	4.9	0.10		
2	ACA [b]	15.4	0.07	5.7	0.71	1.08	0.22
2	(*R*)-camphor	17.99	0.25	7.05	0.55	1.86	0.20
2	(*S*)-camphor	17.99	0.25	7.05	0.55	1.86	0.20

[a] Data refer to the best-fitting model for each compound $\chi^2 < 1.3$. [b] Adamantanecarboxylic acid.

of **2** is correlated with a longer lifetime, since the steady-state fluorescence of the *i*th component can be expressed as $F_i = \alpha_i\tau_i$, where α_i is the molar fraction of fluorophore having the lifetime τ_i: the enhancement of F_{tot} is therefore due to the high population of long-lived components. These results are consistent with the inclusion of fluorescent guests within the β-cyclodextrins:[43] the longer lifetime in the case of inclusion complexes has been attributed to the protection of the excited state from dynamic quenching by water molecules. The present results are consistent with those previously reported for the dansylglycine-β-cyclodextrin, which showed two components with lifetimes of 16.9 and 5.6 ns.[21] The presence of a two well-defined exponential components suggests an equilibrium between two conformations of **2**: one with the dansyl group fully encapsulated, as observed in the solid state, and another one with the dansyl group outside the cavity and thus more exposed to the bulk solvent. This equilibrium is shifted towards the self-included form, as indicated by the molar fraction α_1 (0.9) of the long-lived component. The three different lifetimes obtained from the fluorescence decay experiments for **1** can be attributed to three different rotational isomers of **1** in solution. It is beyond the scope of this work to investigate the photophysical behavior of this molecule in solution. For compound **2** only two components are needed to fit the fluorescence decay. This may be due to the relative large fluorescence associated with the self-included species as compared to that of the short-lifetime component: owing to the small fraction of fluorescence associated with τ_2, we could not resolve the conformational heterogeneity of **2** as well as for **1**.

All the above spectroscopic data strongly suggest that self-inclusion of the dansyl moiety in the cyclodextrin cavity is also the preferred conformation in aqueous solution.

Fluorescence sensing properties: In order to verify whether the structural features of **2** could be correlated to its host–guest sensory properties, analogous to those of previously reported dansyl-modified β-cyclodextrins, we compared the fluorescence intensity of **2** in the absence and presence of various guests of different size and shape, present in excess (Table 7). The most

Table 7. Fluorescence intensities (standard deviations 0.001–0.004) of aqueous solutions of **2** in the absence and presence of copper(II) and with an excess (100:1) of various guests (pH = 8.0, tetraborate buffer:ethanol = 10:1).

Guest	*F*(**2**) (A. U.)	*F*(**2** + Cu^{II}) [a] (A. U.)
–	1.000	1.009
ACA	0.311	0.259
(*R*)-camphor	0.514	0.481
(*S*)-camphor	0.518	0.484
(*S*)-borneol	0.370	0.313
(*R*)-fenchone	0.675	0.637
(*S*)-fenchone	0.673	0.637
cholesterol [b]	0.872	0.822

[a] Cyclodextrin:copper(II) = 1:1. [b] Cyclodextrin:cholesterol = 1:3.

effective guest was found to be adamantanecarboxylic acid (ACA). Its size and tricyclic rigid structure allows it to fit well into the β-cyclodextrin cavity, and it thus form a very stable inclusion complex. Indeed, the fluorescence lifetime distribution changed upon addition of ACA: α_1 for the component having a longer lifetime (τ_1 = 15.4 ns) was smaller than in free **2** (Table 6); this suggests that the fraction of molecules with the dansyl group inside the cavity had decreased. A similar effect was observed in the presence of (*R*)- or (*S*)-camphor. Accordingly, the shorter components have a larger contribution to the total fluorescence; this allows a better resolution of the multiexponential decay. The negative circular dichroism band at 340 nm progressively decreased in intensity upon addition of ACA (Fig. 7). The spectroscopic evidence presented above is

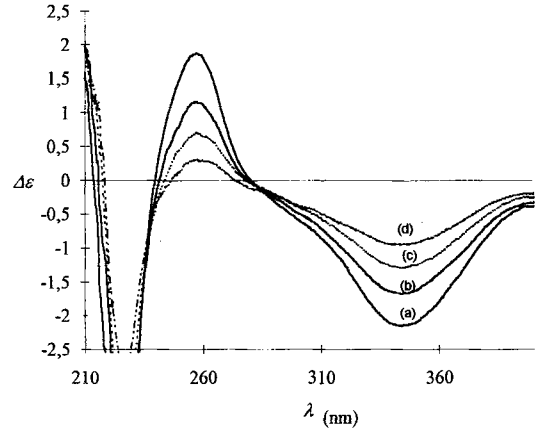

Fig. 7. Circular dichroism spectra of **2** (6×10^{-5} M in H_2O) in the absence (a) and presence of ACA with ACA:**2** ratios of 1:1 (b), 2:1 (c), and 3.5:1 (d) (tetraborate buffer, pH = 8.0).

0947-6539/96/0204-0378 $ 15.00+.25/0 Chem. Eur. J. 1996, 2, No. 4

consistent with an in–out shift of the dansyl moiety induced by the guest, as previously proposed for analogous fluorescent cyclodextrins.

Effect of copper(II) on fluorescence: The copper(II) ion is known to induce fluorescence quenching in DNS amino acids and dipeptides.[44] Recently, we have shown that static quenching occurs in the case of DNS amino acids in the presence of copper(II) complexes. The quenching process is most likely promoted by complexation of the copper(II) ion to a donor group adjacent to the dansyl group, with subsequent abstraction of the sulfonamide hydrogen; coordination of the deprotonated sulfonamidate group to Cu^{II} has been shown to take place both in solution (by polarimetric studies)[45] and in the solid state (X-ray crystal structure analysis).[46]

In the presence of the copper(II) ion at pH = 8, the fluorescence of cyclodextrin **2** underwent only negligible quenching, in contrast to results observed for DNS-en (**1**). This suggests that the lack of quenching effect for **2** was not due to the absence of strongly complexing groups, but rather to conformational constraints. In fact, the inclusion of the dansyl group modifies the conformation of the ethylenediamine linker, so that the two nitrogen atoms are unable to form a chelate ring. Furthermore, the quenching in **1** is strongly dependent upon pH: it is negligible at pH = 7.0 and very large at pH = 8.0 (results not shown). This suggests that the interaction between copper(II) and the dansyl group occurs through the deprotonated sulfonamide nitrogen.

Finally, we evaluated the possibility of enhancing the effect of a guest molecule on the fluorescence of **2** in solution, by addition of the copper(II) ion. The expulsion of the dansyl moiety from the cyclodextrin cavity should allow the complexation of the copper(II) ion by the dansylethylenediamine moiety. This would produce an additional quenching effect. The fluorescence intensities of **2** with various guests in the presence or in the absence of copper(II) are compared in Table 7. Indeed, the ability of **2** to act as a fluorescent optical sensor increases, since the difference between the fluorescence of free **2** and the host–guest complex is greater.

The hydrophobic effect responsible for the formation of the self-inclusion complex is therefore very strong, since it is able to compete with the metal-ion complexation process.

Conclusions

In this work we have described the structural features of a fluorescent sensor based on β-cyclodextrin covalently linked to a dansyl group. For the first time, an inclusion complex of a dansyl moiety within a β-cyclodextrin cavity has been fully characterized both in the solid state and in solution. The present results could serve as a basis for the critical revision of the inclusion models proposed so far to explain the spectroscopic behavior, enantioselectivity, and other chromatographic properties of dansyl derivatives in the presence of β-cyclodextrins.

The crystal structure reveals that β-cyclodextrin is able to fully encapsulate in its cavity a group of appropriate size, such as the DNS group, by folding the arm bridging the β-cyclodextrin and the DNS group. To the best of our knowledge, this is the first example of self-inclusion causing a significant distortion of the heptagonal symmetry of the β-cyclodextrin ring. The elliptical shape of the molecule, with the direction of the longer principal axis almost parallel to the naphthalene plane, is achieved by a series of relatively small distortions in the bond geometry.

A self-inclusion phenomenon has also been observed in 6-deoxy-6-cyclo(L-His-L-Leu)-β-cyclodextrin,[33] but the leucine side chain is only partially encapsulated and the isopropyl group is close to the plane formed by the C(5) atoms. Consequently, no significant distortion of the torus shape of the molecule was induced. The greater degree of encapsulation of the hydrophobic functionality in **2** may partly be attributed to the size of the included group and to the greater flexibility of the linker.

Detailed spectroscopic studies suggested that self-inclusion of the dansyl group was also retained in solution. Two-dimensional NMR showed that the fluorophore was most likely included in the cavity with the H6–H2 axis close to the cyclodextrin axis, in good agreement with the solid-state structure. Furthermore, the correspondence of the structural features derived by NMR and crystallographic studies with circular dichroism models allowed us to validate the existence of a direct relationship between the sign of the CD bands and the orientation of the dansyl group within the cavity.

The tight self-inclusion observed both in the solid state and in solution did not prevent compound **2** from acting as a fluorescent optical sensor for neutral guests. The fluorescence of **2** was not quenched by copper(II), because the the conformation of the ethylenediamine linker prevented the two nitrogen atoms from forming a chelate ring. In the presence of a suitable guest, the expulsion of the DNS group increased the degrees of freedom of the linker, allowing the coordination of copper(II) and hence fluorescence quenching. It is therefore possible, as a general strategy, to exploit conformational changes induced by the displacement of the aromatic moiety to enhance the sensitivity of the optical signal produced and thus to produce a more efficient fluorescent sensor.

Experimental Section

The following materials were used: β-cyclodextrin hydrate (Janssen), dansyl chloride (Sigma), and ethylenediamine (Janssen). The latter reagent was distilled prior to use. TLC was conducted on precoated silica gel plates (60 F-254, Merck); detection of cyclodextrin derivatives was achieved by UV irradiation (λ = 254 nm) or by using the anisaldehyde test. IR spectra were measured with a Perkin-Elmer 298 spectrometer. All IR spectra were recorded at room temperature from $\tilde{\nu}$ = 4000 to 600 cm^{-1}. CI and FAB mass spectra were obtained on a Finningan MAT SSQ 710 (CG-MS) mass spectrometer. Melting points were measured on an electrothermal melting-point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained on Bruker AMX 400 (400 MHz), AC 300 (300 MHz), or AC 100 (100 MHz) spectrometers. ^1H and ^{13}C chemical shifts in D_2O were measured with the sodium salt of 3-trimethylsilylpropionic acid as external reference. Optical rotations were recorded on a Autopol II (Rudolph Research) polarimeter with a sodium lamp (589 nm). UV spectra are obtained with a Kontron Uvicon 860 spectrophotometer. Fluorescence spectra were measured with a JASCO FP 770 instrument in 0.2 × 1 cm quartz cell. CD spectra were recorded with a JASCO J-500 A spectropolarimeter.

Dansylethylenediamine (**1**): A solution of dansyl chloride (3.67 g, 13.6 mmol) in CH_3CN (360 mL) was added at 0 °C to ethylene diamine (9.1 mL, 136 mmol) with stirring. The mixture was then allowed to stir at room temperature for 3 h. Unreacted dansyl chloride was removed by acidification (pH = 4) and extraction with diethyl ether. The aqueous layer was basified with aqueous NaOH and extracted with $CHCl_3$. The extract was dried over Na_2SO_4, concentrated, and dried in vacuo to provide 2.9 g (73%) of **1** as a solid: M.p. 135–137 °C; ^1H NMR (100 MHz, $CDCl_3$, TMS): δ = 1.70 (brs, 2H; NH_2), 2.68 (m, 2H; CH_2N-SO_2), 2.88 (m, 2H; CH_2NC), 2.89 (s, 6H; $N(CH_3)_2$), 7.18 (d, ^3J(H,H) = 7.6 Hz, 1H; arom. H6), 7.55 (m, 2H; arom. H7, H3), 8.28 (m, 2H; arom. H2, H8), 8.55 (d, ^3J(H,H) = 7.9 Hz, 1H; arom. H4); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 40.92 (CH_2), 45.41 (CH_3), 45.55 (CH_2), 115.24 (CH arom.), 118.81 (CH arom.), 123.19 (CH arom.), 128.36 (CH arom.), 129.55 (CH arom.), 129.67 (C_{quat} arom.), 129.96 (C_{quat} arom.), 130.39 (CH arom.), 134.90 (C_{quat} arom.), 152.04 (Cquat arom.); IR (KBr): $\tilde{\nu}$ = 3390 (NH), 3050 (CH arom), 2970 (CH aliph.), 2800 (CH aliph.), 1590 (S=O asymm.), 1330, 1150 (S=O symm.), 1110 cm^{-1}; MS (CI, CH_4): m/z (%): 294 (100) [M + 1$^+$], 293 (33) [M^+], 235 (14) [$(CH_3)_2N$-$C_{10}H_6$-SO_2-H^+], 218 (8) [$(CH_3)_2N$-$C_{10}H_6$-SO^+], 171 (19) [$(CH_3)_2N$-$C_{10}H_6$-H^+].

6-*O*-(*p*-Tosyl)-β-cyclodextrin (CDOTs) and **6-deoxy-6-iodo-β-cyclodextrin** (CDI) were synthesized according to a literature procedure [30].

6-deoxy-6-*N*-(*N'*-(5-dimethylamino-1-naphthalenesulfonyl)diaminoethane)-β-cyclodextrin (2): Dried CDI (0.150 g, 0.12 mmol) was dissolved in anhydrous DMF (11 mL), and **1** was added (CDI:**1** molar ratio = 1:20). The reaction was carried out at 50 °C under nitrogen. After 3 d the DMF was evaporated in vacuo at 40 °C. The yellow syrup obtained was washed with acetone. The solid obtained was dissolved in the minimum amount of water and precipitated again with acetone. The precipitate collected by suction was dissolved in water and the solution was applied to a column of CM-Sephadex C-25 resin (in NH_4^+ form). The column was eluted initially with water (120 mL) and then with increasing concentrations of aqueous ammonium hydrogen carbonate from 0 to 0.2 M (800 mL, total volume). The collected fractions were assayed by TLC. Fractions that gave only one spot with $R_f = 0.7$ (eluent 5:3:1 propanol:water:ammonia) were combined and evaporated to dryness at 40 °C in vacuo to decompose ammonium hydrogencarbonate. The residue was a yellow powder (yield, 57% based on CDI). The purity was checked by HPLC on a Lichrosorb-NH_2 column (4.6 × 250 mm, 10 μm) and with a mixture of CH_3CN and water (6:4) as eluent. Crystals of the product for X-ray diffraction were obtained by dissolving the powder in the minimum amount of water at 40 °C and allowing the solution to cool slowly first to room temperature, and then to 4 °C.

2: M.p. 117 °C (decomp.); $[\alpha]_D^{21} = 130$ (c = 0.7, D_2O); ^1H NMR (400 MHz, D_2O, 37 °C): δ = 2.09 (m, 1H; H14), 2.10 (d, ^2J(H,H) = 12.7 Hz, 1H; H(6)1), 2.30 (m, 1H; H14), 2.42 (dd, ^3J(H,H) = 11.3 Hz, ^2J(H,H) = 12.2 Hz, 1H; H(6)1), 2.52 (d, ^3J(H,H) = 9.36 Hz, 1H; H(5)5), 3.12 (s, 6H; $N(CH_3)_2$), 3.23 (m, 1H; H13), 3.43 (m, 1H; H13), 3.12–4.47 (m, 41H; cyclodextrin protons, see Table 4 for full assignment), 4.94 (d, ^3J(H,H) = 3.6 Hz, 1H; H(1)5), 4.99 (d, ^3J(H,H) = 3.5 Hz, 1H; H(1)2), 5.00 (d, ^3J(H,H) = 3.1Hz, 1H; H(1)1), 5.18 (d, ^3J(H,H) = 3.4 Hz, 1H; H(1)6), 5.19 (d, ^3J(H,H) = 3.1 Hz, 1H; H(1)4), 5.32 (d, ^3J(H,H) = 3.2 Hz, 1H; H(1)3), 5.34 (d, ^3J(H,H) = 3.3 Hz,1H; H(1)7), 7.73 (d, ^3J(H,H) = 7.4 Hz, 1H; H6), 7.74 (t, ^3J(H,H) = 6.4 Hz, 1H; H3), 8.16 (t, ^3J(H,H) = 8.4 Hz, 1H; H7), 8.37 (d, ^3J(H,H) = 8.7 Hz, 1H; H8), 8.53 (d, ^3J(H,H) = 7.2 Hz, 1H; H2), 8.90 (d, ^3J(H,H) = 8.4 Hz, 1H; H4); ^{13}C NMR (300 MHz, D_2O, 37 °C): δ = 44.91 (CH_2N), 49.02 (CH_3), 49.48 (CH_2N), 51.84 (CH_2N), 61.87, 62.45, 63.01, 63.14, 63.28 (C(6)), 73.05, 74.14, 74.34, 74.55, 74.64, 74.80, 75.01, 75.28, 75.33, 75.42, 75.50, 76.05, 76.14, 76.22, 76.45 (C(3), C(5), C(2)), 83.22, 83.39, 83.56, 83.70, 83.92, 84.54, 87.58 (C(4)), 103.95, 104.09, 104.85, 104.93, 105.31, 105.45, 105.54 (C(1)), 118.98 (CH arom.), 120.65 (CH arom.), 126.66 (CH arom.), 131.54 (C_{quat} arom.), 132.38 (C_{quat} arom.), 132.99 (CH arom.), 133.16 (CH arom.), 133.39 (CH arom.), 138.15 (C_{quat} arom.), 156.43 (C_{quat} arom.); IR (KBr): $\tilde{\nu}$ = 3700–2800 (br, OH), 2940 (CH), 1630, 1580 (S=O asymm.), 1400, 1320, 1150 (S=O symm.), 1020 cm^{-1}; MS (FAB) m/z: 1411 [M + 1$^+$]; Anal. (after drying under vacuum): $C_{56}H_{87}N_3O_{36}S \cdot 9H_2O$ (1572.5): calcd C 42.77; H 6.73; N 2.67; found: C 42.98; H 6.45; N 2.26.

2D NMR spectra: COSY and TOCSY spectra (2 K, 512 experiments) were obtained at 310 K with the pulse programs COSYTP and MLEVTP supplied by Bruker, with TPPI phase cycling. ROESY spectra (2 K, 512 experiments) were obtained at 310 K using States phase cycling with 680 Hz downfield offset irradiation during spin-lock, in order to minimize scalar effects, with a 250 ms mixing time. Assignment of the cross-peaks in the ROESY spectra was not unambiguous for aromatic protons H3 and H6, since they overlap partially. A ROESY spectrum at lower temperature (293 K), at which the two signal are better separated, showed the occurrence of cross-peaks for proton H3 and no cross-peaks for H6, except for with the methyl protons H11 and H12.

Crystal structure: X-Ray study was performed using a graphite monochromated $Cu_{K\alpha}$ radiation and a pulse-height discrimination on a CAD4 Turbo Enraf-Nonius diffractometer equipped with a Micro VAX3100 server of the "Centro di Studio di Biocristallografia del CNR" at the University of Naples. Transparent, pale green prismatic crystals of **2** were obtained from a saturated aqueous solution. Preliminary oscillation and Weissenberg photographs were taken to establish the crystal symmetry and the space group. Unit cell parameters were obtained by a least-squares procedure on the angular parameters of 25 reflections in the θ range of 21–29°. A summary of the crystallographic data is given in Table 8.

Intensity data were collected in the ω–2θ scan mode, with a scan angle of $\Delta\omega = (1.1 + 0.16\tan\theta)$; background counts were taken in an additional area of $\Delta\omega/4$, on both sides of the main scan, using the same scan speed. Prescan runs were made at a speed of 5° min^{-1}. Reflections with a net intensity $I > 0.5\sigma(I)$ were measured at lower speed depending on the $\sigma(I)/I$ value, in the range 1–4° min^{-1}. Two intensity-control reflections were measured every 60 min of X-ray exposure time in order to monitor the crystal and the electronic stability; no significant change in intensity was observed during data collection. Orientation matrix checks were made with respect to the scattering vectors of two well-centered reflections every 400 reflections measured. All reflections were corrected for Lorentz and polarization effects. 8266 reflections were collected in a θ range of 1 to 70°; 6188 had a net intensity greater than $3.0\sigma(I)$, and were considered observed and used for further calculation. The structure was solved by application of direct methods included in the crystallographic package SIR92 [47]. The direct phase expansion procedure of the best solution led to a molecular fragment containing all non-hydrogen atoms. Subsequent difference Fourier analysis revealed 16 cocrystallized water molecules. The hydrogen atoms were introduced in their stereochemically expected positions with an isotropic temperature factor equal to the B_{eq} of the heavy atom to which they are linked. Their parameters were kept fixed. Those hydrogen atoms linked to primary hydroxyl groups and to the water molecules were not included in structure factor calculations. No statistical occupancy for solvent oxygen atoms was observed. The structure was refined using the Molen package [48]. Full-matrix least-squares procedure was used, minimizing the quantity $\sum w(F_o - F_c)^2$ with weights w equal to $1/\sigma^2(F_o)$, and converged to a final R factor of 0.059 and Rw of 0.059 using anisotropic temperature factors for the non-hydrogen atoms. Refinement was ended when the shifts in the atomic coordinates and temperature factors were less than 1/5 and 1/3 of the corresponding standard deviations, respectively. Atomic scattering factors for all atomic species were calculated from Cromer and Waber [49]. In the Supplementary Material the final atomic parameters are reported.

Table 8. Crystal Data of **2**·16H_2O.

molecular formula	$C_{56}H_{87}N_3O_{36}S \cdot 16H_2O$
M_r	1698.69
crystal system	orthorombic
space group	$P2_12_12_1$
Z	4
a (Å)	27.293(8)
b (Å)	17.801(2)
c (Å)	15.881(4)
V (Å^3)	7716(3)
ρ_{calcd} (g cm^{-3})	1461
radiation (λ in Å)	$Cu_{K\alpha}$ (1.5418)
measured reflections	8266
observed reflections (with $I > 3\sigma(I)$)	6188
R	0.059
Rw	0.059
no. parameters refined	1010
T (K)	295

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-1220/1. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: Int. code +(1223)336-033 or e-mail: teched@chemcrys.cam.ac.uk).

Fluorescence measurements: Concentrated stock solution of **2** (6×10^{-3} mol L^{-1}), **1** (6×10^{-3} mol L^{-1}) were prepared at pH = 8 in a 0.1 M aqueous tetraborate buffer. Solutions and various guests (60×10^{-3} mol L^{-1}) were prepared in ethanol. The aqueous solution of $CuCl_2$ had a concentration of 6×10^{-3} mol L^{-1}. Solutions for fluorescence measurements were prepared by diluting standard solutions of **1**, **2**, and **2**/$CuCl_2$ (1:1) to a final concentration of 6×10^{-5} mol L^{-1}; 0.5 mL of these solutions were transferred to the cell and measured. Then 50 μL of a concentrated solution of guest or of ethanol was added with a Hamilton 100 μL syringe. Fluorescence intensity corresponding to a maximum emission intensity was used instead of the area, since all samples showed the same peak shape and maxima. Alternate measurements of samples and of a reference solution of **2** (6×10^{-5} mol L^{-1}) were performed at 25 °C, in order to compensate for lamp fluctuations. Ten measurements of each sample and of the reference were made for each point. The fluorescence intensity of all the samples (F_i) was corrected, according to the expression: $(F_i)_{corr} = (F_i)/F_{ref}$, where (F_i) is the observed fluorescence intensity and F_{ref} is the intensity of the reference solution of **2**, both measured at the same excitation and emission wavelength (corresponding to maximum fluorescence). In each measure, the corrected fluorescence intensity was normalized for F_o, the fluorescence of the fluorophore in the absence of any guest or metal ion, at the same concentration and in the same solvent (tetraborate buffer:ethanol = 10:1) as all other samples.

Time-resolved experiments: Lifetimes were measured on a single-proton counting instrument equipped with a nanosecond pulse flash lamp (Edimburg instrument, mod. F199), modified in order to allow N_2 flux at a rate of 1 L min^{-1}; Jasco and Farrand monochromators and Philips XP20202Q photomultiplier were used for fast detection. Fast NIM electronics were from EG & G, Tennelec and Silenox. Decays were recorded on a Silena BS27n multichannel analyser (512 channels). Alternate measurements of the sample and of the scattering solution (glycogen) were performed in order to compensate for lamp fluctuations and drift. The excitation wavelength was set at 350 nm, and measurements on each sample were recorded at different wavelengths in order to cover the emission spectrum. Deconvolution of the decay profile from the lamp profile was carried out, and fluorescence lifetimes were calculated from decay data, utilizing a global approach [50] by means of two- or three-lifetime models, as previously reported [51]. For **1** and **2** in the presence of guests the fluorescence decay was analyzed using a three exponential decay model; a more simple model gave worse chi-square values. For compound **2** in the absence of guests a two exponential decay function allowed us to obtain a chi-square lower than 1.3.

CD measurements: A solution of **2** (0.1×10^{-3} mol L^{-1}) was obtained by dilution of freshly prepared concentrated solution (1×10^{-3} mol L^{-1}). For the measurements

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in the presence of guests, aliquots of a concentrated solution (1×10^{-2} mol L^{-1} in ethanol) of the guest (ACA) were added to 2.5 mL of a solution of **2** to make up a final guest concentration of 1×10^{-3} mol L^{-1} (solution in 10% ethanol).

Acknowledgments: We thank the Centro Interfacoltà di Misure of the University of Parma for making available the CD and NMR facilities utilized for the present study. The present work was supported by a grant from CNR (Rome)-Progetto Chimica Fine e Secondaria II and from the Italian Ministero per la Ricerca Scientifica e Tecnologica (MURST).

Received: August 18, 1995 [F191]

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