Difunctionalized β-cyclodextrins: synthesis and X-ray diffraction structure of $6^{I},6^{II}$-dideoxy-$6^{I},6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose †

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Received (in Cambridge, UK) 3rd January 2001, Accepted 3rd April 2001
First published as an Advance Article on the web 2nd May 2001

The synthesis, solution NMR investigation and solid-state structural characterization of a new difunctionalized β-cyclodextrin (β-CD) are reported. $6^{I},6^{II}$-Dideoxy-$6^{I},6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose is synthesized for the first time using a regioselective synthetic procedure. On the basis of an aqueous solution NMR investigation, the intramolecular interaction of the two pyridine rings with the upper rim of the cavity is proposed. $6^{I},6^{II}$-Dideoxy-$6^{I},6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose, $C_{56}H_{86}N_4O_{33}$, crystallizes in the monoclinic $P2_1$ space group with cell dimension $a = 26.303(5)$, $b = 15.670(5)$, $c = 8.276(2)$ Å and $\beta = 103.60(2)°$, and 5.5 molecules of water for each independent β-cyclodextrin molecule. The structure refines to $R = 0.103$ for 2270 observed reflections [$I \geq 3\sigma(I)$] and represents the first example of a complete structural characterization of a branched difunctionalized β-cyclodextrin. In the solid state, the macrocycle structure maintains an approximate seven-fold symmetry with only small changes occurring in the cyclic carbohydrate conformation where two consecutive primary hydroxy groups are substituted with bulky moieties. The two aminoethylpyridine groups linked to contiguous glucosidic units show different behaviour, with one group extending away from the cavity of the β-CD, the other remaining in the proximity of the 'mouth' of the cavity. However, in the crystal the aminoethylpyridine group extending away from the cavity of the β-CD is deeply inserted into the cavity of the adjacent β-CD molecule translated along the *c* axis, giving rise to long rows of β-CD molecules stabilized by these host–guest interactions. The resulting polymeric arrangement has already been observed in crystal structures of other monosubstituted β-CDs.

Introduction

Cyclodextrins (CDs) are truncated, cone-shaped, cyclic oligosaccharides composed of six or more α-1,4-linked glucose moieties.[1–3] CDs present a hydrophobic cavity with their primary hydroxy groups on the narrow side of the macrocycle and the secondary hydroxy groups on the opposite side.[3–6] They can function as chiral hosts, and for this behaviour they have been widely studied as receptors for a large variety of molecules[2,7–9] and as molecular carriers.[1,2,8] Furthermore, CDs have received considerable attention as models mimicking the behaviour of biological macromolecules.[2,10]

CDs possess as functional groups only hydroxy groups. Consequently, the introduction of other functional groups on their skeleton can modify and possibly improve some of their features, such as solubility, stability and selectivity, when forming inclusion complexes.[1,2,8] Among known CDs, β-CDs (or cyclomaltoheptaoses), have been the object of many investigations. A large number of monofunctionalized β-CDs have been described and characterized in solution[11–16] as well as in the solid state[17–21] and their applications as metal enzyme models or chiral discriminating agents, as well as their inclusion and catalytic abilities have been investigated.

Substitution of more OH groups at desired positions by different chemical functions can lead to multisite recognition systems, in which the hydrophobic cavity and different substituent groups as recognition elements can be involved. Therefore selectivity and enantioselectivity can be greatly increased and modulated.[22–25] A few 6-difunctionalized cyclodextrins have already been described as molecular receptors[22,23,26] or models for the mechanism of action of biomolecules.[27–32] In particular, the functionalization by metal-ion-complexing groups has led to systems of undiscussed interest.[12–14,28,33–36] Ferredoxin,[30] haemoglobin-like,[27,29] carbonic anydrase,[32] superoxide dismutase (SOD),[34] and P-450 cytochrome models[37] have been built using difunctionalized CDs. Recently, the crystal structure of $6^{I},6^{II}$-diamino-$6^{I},6^{II}$-dideoxy-β-cyclomaltoheptaose and its complex with platinum has been reported by us.[38,39] They represent the only examples of difunctionalized β-cyclodextrins structurally characterized by single-crystal X-ray diffraction and NMR. The molecule is able to complex a metal ion, and shows chiral recognition ability[33] towards amino acids. The functionalized cyclodextrins show different properties with respect to other regioisomers.[33] This suggests that the

† Electronic supplementary information (ESI) available: the Cremer and Pople puckering parameters of the glucose residues and hydrogen-bond parameters. See http://www.rsc.org/suppdata/p2/b1/b100126o/

 DOI: 10.1039/b100126o

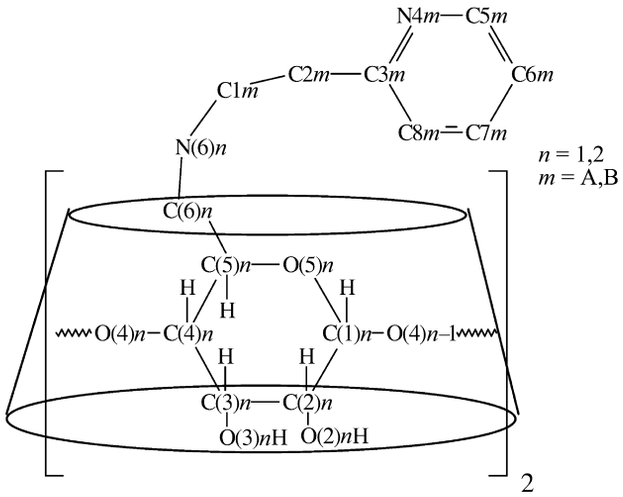

Fig. 1 Schematic representation of the $6^{I},6^{II}$-dideoxy-$6^{I},6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose molecule with numbering of the atoms.

improvement in the structural and conformational properties on more sophisticated difunctionalized β-CDs can represent a very important step in the rational design of this class of compounds.

Here, we report the synthesis and the first structural characterization by X-ray diffraction analysis of a branched difunctionalized β-cyclodextrin, namely $6^{I},6^{II}$-dideoxy-$6^{I},6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose (ABAEPY) (Fig. 1).

Experimental

β-Cyclodextrin was purchased from Fluka; anhydrous *N*,*N*-dimethylformamide was purchased from Aldrich. They were used without further purification. Thin layer chromatography (TLC) was carried out on silica gel plates (Merck 60-F254). β-CD derivatives were detected on TLC by UV and by the anisaldehyde test. A Merck Lichroprep RP-8 column (40–63 μm) was used for reversed-phase column chromatography.

Synthesis

A solution of $6^{I},6^{II}$-dideoxy-$6^{I},6^{II}$-diiodo-β-cyclomaltoheptaose[40] (0.1 g) in 2-(2-aminoethyl)pyridine (AEPY) (1 ml) was stirred under nitrogen at 70 °C. After 12 h, the reaction mixture was added to 200 ml of acetone under stirring, and the solid obtained was collected by filtration and washed with acetone. The crude product was purified using a CM Sephadex C-25 column (20 × 600 mm, NH_4^+-form). A linear gradient 0–0.2 M of aq. NH_4HCO_3 (400 ml) was used as the eluent. The appropriate fractions were combined ($R_f = 0.45$, 5 : 3 : 2 : 1, PrOH–H_2O–AcOEt–NH_3) to give ABAEPY (0.03 g, 30%), mp 205 °C (decomp.); CD (H_2O) $\lambda = 218$ nm, $\Delta\varepsilon = 0.139$; $\lambda = 241$ nm, $\Delta\varepsilon = -0.117$; $\lambda = 261$ nm, $\Delta\varepsilon = 0.12$; FAB MS *m*/*z* 1343 (M + H); ^{1}H NMR (D_2O; 499.88 MHz) δ 8.43 [d, 1H, H-6 of A pyridine (py) ring, $J = 5$Hz], 8.39 (d, 1H, H-6 of B pyridine ring, $J = 4.5$ Hz), 7.77 (m, 2H, H-4 of py), 7.32 (m, 2H, H-3 of B py and H-5 of A py ring), 7.26 (m, 1H, H-5 of B py), 7.23 (d, 1H, H-3 of A py), 5.02–4.90 (m, 7H, H-1 of CD), 4.00–3.66 (m, 36H, H_2-6, H-5, -4, -3, -2 of CD), 3.37 (m, 2H, H-4^I and H-4^{II} of CD), 3.20 (d, 1H, H-6^{aI}), 3.15–2.85 (m, 11H, H-6^{aII}, -6^{bI}, -6^{bII} and methylenes of AEPY chains); ^{13}C NMR (D_2O; 50.3 MHz) δ 161.4 (C-2 of py), 151.1 (C-6 of py), 140.7 (C-4 of py), 126.5 (C-5 of py), 124.9 (C-3, -5 of py), 104.6 and 104.2 (C-1 of CD), 86.0 (C-4^I, 4^{II}), 83.5 (C-4 of CD), 79.2–72.0 (C-2, -3, -4 of CD), 72.0 (C-5^I, 5^{II}), 64.1, 63.4, 62.2, 61.9 (C-6 of CD), 51.9, 51.6, 51.3, 51.1 (C-6^I, -6^{II}, CH_2 in AEPY β to py), 39.2 and 38.6 (CH_2 in AEPY α to py) (Calc. for $C_{56}H_{86}N_4O_{33}\cdot 4H_2O$: C, 47.5; H, 6.6; N, 3.9. Found: C, 47.9; H, 6.8; N, 4.1%).

Table 1 Crystal data and structure refinement parameters

Empirical formula	$C_{56}H_{86}N_4O_{33}\cdot 5.5H_2O$
Crystal size	0.15 × 0.13 × 0.06 mm
Crystal system	Monoclinic
Space group	$P2_1$
Unit-cell dimensions	$a = 26.303(5)$ Å
	$b = 15.670(5)$ Å, $\beta = 103.60(2)°$
	$c = 8.276(2)$ Å
Volume	3315(1) Å^3
Z	2
Relative molecular mass	1343.30 + 99.09 Da
Density (calculated)	1.444 g cm^{-3}
Absorption coefficient	0.913 mm^{-1}
Wavelength/Temperature	1.54178/295(2) K
Attenuators	Zr foil (factors: 3.0, 8.7, 25.4)
Take-off angle	6.0°
Detector aperture	6.0 mm horizontal; 6.0 mm vertical
Crystal-to-detector distance	40 cm
Scan type	ω–2θ
Scan width	1.31 + 0.30 tan(θ)
Index ranges	$0 \leqslant h \leqslant 30; 0 \leqslant k \leqslant 17; -9 \leqslant l \leqslant 9$
Reflections collected	5717
Independent reflections	5451
Observed reflections [$I \geq 3\sigma(I)$]	2270
Refinement method	Full-matrix least squares
Goodness-of-fit on *F*	1.552
Final *R* indices [$I \geq 3\sigma(I)$]	$R = 0.103$, $wR = 0.110$
Largest diff. peak and hole	0.471 and −0.413 e Å^{-3}

NMR Analysis

^{1}H NMR spectra were recorded at 25 °C in D_2O with a Varian Inova 500 spectrometer at 499.883 MHz. The ^{1}H NMR spectra were measured by using standard pulse programs from the Varian library. In all cases the length of the 90° pulse was ≈7 μs. 2D experiments were acquired using 1k data points, 256 increments and a relaxation delay of 1.2 s. Transferred nuclear Overhauser effect (TROESY) spectra were obtained using a 300 ms spin-lock time. ^{13}C NMR spectra were recorded at 25 °C in D_2O with a Bruker AC-200 spectrometer at 50.9 MHz. Sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) was used as external standard.

X-Ray diffraction analysis‡

Colourless crystals of ABAEPY in the form of thin plates were obtained at room temperature by slow evaporation of an aqueous solution over a period of three weeks. Using different vapour-diffusion techniques, other crystallization attempts were made, but failed to give larger and better-quality crystals. A crystal of dimensions 0.15 × 0.13 × 0.06 mm was used for X-ray diffraction analysis. Unit-cell parameters were determined by least-squares refinement of the setting angles of 25 carefully centred reflections in the range $15° < \theta < 25°$. A summary of crystallographic data is reported in Table 1. All measurements were made on a Rigaku AFC5R diffractometer with graphite-monochromated CuKα radiation and a 12 kW rotating anode generator. The data were collected at room temperature using the ω–2θ scan technique to a maximum 2θ-value of 124.2°. The weak reflections [$I > 30.0\sigma(I)$] were rescanned (maximum of 4 rescans) and the counts were accumulated to assure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak to background counting times was 2 : 1. The diameter of the incident beam collimator was 0.5 mm and the crystal-to-detector distance was 400.0 mm.

A total of 5451 independent reflections were measured and corrected for Lorentz and polarization factors. Of these, only 2270 reflections were considered as observed, having net intensity $I \geq 3.0\sigma(I)$. No absorption correction was applied.

‡ CCDC reference number 149296.

Table 2 a) Selected torsion angles (°) describing the linkage bonds between the glucose residues and the orientations of the primary hydroxy group; b) the conformation of the aminoethylpyridine moieties

a)

	G1	G2	G3	G4	G5	G6	G7
C(3)*n*–C(4)*n*–O(4)*n*–C(1)*n* + 1	100.3	131.9	116.5	131.7	130.6	105.5	147.6
C(5)*n*–C(4)*n*–O(4)*n*–C(1)*n* + 1	−142.2	−106.9	−121.6	−110.1	−108.3	−134.9	−91.9
O(5)*n*–C(1)*n*–O(4)*n* − 1–C(4)*n* − 1	118.3	113.0	121.6	108.8	120.2	112.8	102.5
C(2)*n*–C(1)*n*–O(4)*n* − 1–C(4)*n* − 1	−115.2	−126.7	−117.0	−129.6	−119.0	−126.7	−135.8
O(5)*n*–C(5)*n*–C(6)*n*–O(6)*n*			69.0	−67.2	−71.4	61.5	54.7
			−70.7				−61.7
C(4)*n*–C(5)*n*–C(6)*n*–O(6)*n*			−170.4	53.2	49.6	−171.7	174.9
			49.9				58.4
O(5)*n*–C(5)*n*–C(6)*n*–N(6)*n*	60.9	59.7					
C(4)*n*–C(5)*n*–C(6)*n*–N(6)*n*	179.9	−174.0					

b)

	Angle/°		Angle/°
C(5)1–C(6)1–N(6)1–C1A	36.9	C(5)2–C(6)2–N(6)2–C1B	78.5
C(6)1–N(6)1–C1A–C2A	65.3	C(6)2–N(6)2–C1B–C2B	173.0
N(6)1–C1A–C2A–C3A	78.3	N(6)2–C1B–C2B–C3B	18.1
C1A–1C2A–C3A–N4A	62.9	C1B–C2B–C3B–N4B	−87.0
C1A–C2A–C3A–C8A	−113.2	C1B–C2B–C3B–C8B	99.4

Table 3 Geometrical data

Residue	Radius/Å [a]	Distance/Å [b]	Angle/° [c]	Tilt angle/° [d]	Planarity [e]
G1	4.81	4.31	129.1	22.8	−0.26
G2	5.08	4.30	124.8	3.3	−0.02
G3	5.00	4.47	131.2	8.5	0.24
G4	5.16	4.28	129.3	4.8	−0.04
G5	5.28	4.43	125.4	15.7	−0.25
G6	5.02	4.28	128.9	21.7	0.18
G7	4.79	4.46	129.4	10.1	0.15

[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 made by 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 RMS deviation of each O(4)*n* atom from the least-squares plane of the seven O(4) atoms.

The structure was solved by a straightforward application of the phase-determination procedure, using the SIR92[41] program. The best *E*-map revealed most of the non-hydrogen atoms, and the oxygen atoms of some co-crystallized water molecules. Subsequent difference electron-density maps revealed the positions of the remaining atoms, and of water molecules. A statistical disorder in the positions of the O(6)3 and O(6)7 primary hydroxy groups was also observed.

The structure was refined using full-matrix least-squares procedures as programmed in SDP Package[42] on F_o-values. The refinement was carried out on 569 parameters, including atomic coordinates and anisotropic thermal factors only for the heavy atoms of the two functionalized moieties and the C(6)*n* and O(6)*n* atoms. Isotropic thermal factors were used for the rest of the atoms, including water oxygens. All hydrogen atoms were included in their stereochemically expected positions with thermal factors equal to the equivalent *U* of the carrier atom, except those of the water molecules. The occupancy factors for the statistical O(6)3 and O(6)7 atoms were refined and their final values are 0.6 and 0.4 for O(6)3a and O(6)3b, and 0.5 and 0.5 for O(6)7a and O(6)7b, respectively. The refinement of the occupancy factor of water molecules indicated a total of 5.5 water molecules per β-CD molecule distributed over 7 sites, 4 of which were refined with full occupancy and the remaining 3 with a site occupancy factor of 0.5. Scattering factors were taken from the International Tables for X-Ray Crystallography.[43] All calculations were carried out at the Biocrystallography Research Centre of the CNR at the University of Naples, using a Digital MicroVax 3100 workstation. The structure was refined to final indices $R = 0.103$ and $wR = 0.110$ (Table 1), which somehow reflect the poor quality of the crystal. Selected torsion angles, describing the conformation of the β-CD macrocycle and that of pyridylethylamino moieties, and the geometrical data relative to the cyclodextrin ring, are given in Tables 2 and 3 respectively. The atoms of the glucose residues are indicated as C(*m*)*n* or O(*m*)*n* where *m* denotes the m^{th} atom within the n^{th} glycosidic residue Gn. In Fig. 1 a representation of the molecule is shown with the atom labelling of the first glycosidic residue and of the pyridylethylamino moieties. The Cremer and Pople puckering parameters of the glucose residues and hydrogen-bond parameters are available in the Supplementary information.

Results and discussion

Synthesis of $6^{I},6^{II}$-dideoxy-$6^{I},6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose

ABAEPY was synthesized starting from the appropriate sulfonate using the regioselective method reported by Tabushi.[44] The reaction of 2-(2-aminoethyl)pyridine with the intermediate product $6^{I},6^{II}$-dideoxy-$6^{I},6^{II}$-diiodo-β-cyclomaltoheptaose gave ABAEPY, which was isolated by column chromatography and characterized by NMR and CD spectroscopy.

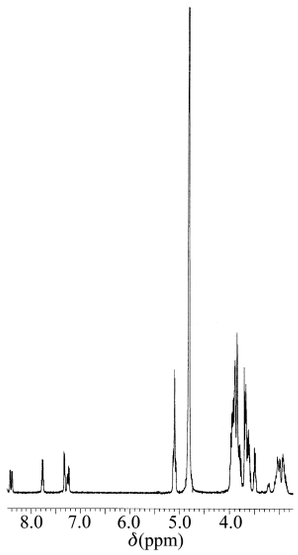

Fig. 2 ^{1}H NMR spectrum (D_2O; 500 MHz) of $6^I,6^{II}$-dideoxy-$6^I,6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose.

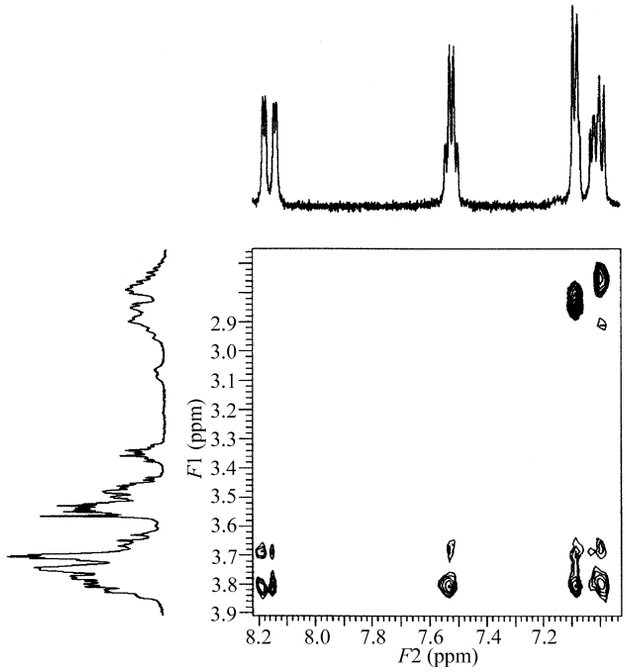

Fig. 3 Contour plot of a portion of the TROESY spectrum (D_2O; 500 MHz) of $6^I,6^{II}$-dideoxy-$6^I,6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose.

NMR analysis

An NMR investigation was carried out to characterize the difunctionalized cyclodextrin. The NMR spectra are not concentration dependent. The ^{1}H NMR spectrum of ABAEPY (Fig. 2) was assigned by the COSY and TOCSY spectra,§ and the ^{13}C NMR data were assigned by HSQC and HSQCTOCSY spectra.§ In the ^{1}H spectrum, the main effect due to the functionalization is clearly observed in the region of anomeric protons, which appear as various signals, as shown by this class of derivatives.[40] Furthermore the functionalization induces an upfield shift of the protons of the modified glucose rings (I and II rings) with respect to the unmodified ones. The 6-H of I and II rings are diastereotopic, as can be seen in the COSY and TOCSY spectra. The protons H-6^{aI}, H-6^{aII}, H-6^{bI}, H-6^{bII} resonate at about δ 3.2, 3.08, 3.06, 2.94 respectively, overlapped by the signals of the ethylenic chains of 2-aminoethylpyridine (AEPY) (chemical shift read on the TOCSY spectrum). Two different group of signals for the pyridine (py) rings H-6, H-3, H-5 atoms can be seen on the ^{1}H spectrum. The signals at δ 7.3 are assigned to the H-5 proton of the ring of one py (A) and to the H-3 proton of the other py ring (B). The multiplet at $\delta \approx 7.2$ is assigned to the H-5 of B py ring and to the H-3 of A py ring. One of the pyridine rings shows the H-3 proton down-shifted with respect to the H-5 proton.

TROESY spectra were carried out in order to investigate the disposition of the two substituents with respect to the β-CD cavity. Dipolar correlations between the aromatic protons and the region of β-CD protons (Fig. 3) suggest intramolecular interaction(s) between aromatic rings and the cavity. On the basis of HSQC, the correlations seem to involve the H-6 and H-5 of β-CD protons, thus suggesting an interaction with the upper rim. The two rings disposed on the upper rim are not deeply included and they lie more or less perpendicular to the cavity. Corey–Pauling–Koltum (CPK) models and the low induced Cotton effect on the CD spectra of ABAEPY in aqueous solution confirm this hypothesis. A stacking interaction of the two aromatic rings can be suggested on the basis of the differences in chemical shifts of the two py rings which can result from the mutual interaction of these rings. The shape of the CD spectrum confirms the proximity between the two pyridine rings. Transition dipole coupling takes place and an excitonic coupling band is thus generated in the CD spectrum at about 250 nm. This has been reported for other systems.[45] The ring current of one py ring could induce the observed shielding of the H-5 and H-6 and deshielding of the H-3 aromatic protons of other ring.

In the ^{13}C spectra, together with signals typically observed for cyclodextrins, we observed the shift due to the functionalization and the non-equivalences of C-6^I and C-6^{II}. Furthermore, in the HSQC spectra the spreading of the signal for H-6 of the unfunctionalized rings can be seen (through C-6 correlation). In particular both protons of a C-6 are shifted in the H-2, -4 region. It is likely that the 6-methylene of the glucopyranosidic rings adjacent to the functionalized rings undergoes shielding due to current effect of the pyridine rings interacting with the upper rim.

Solid-state conformation of β-CD molecule

A stereo view of the molecular structure of ABAEPY as obtained by X-ray diffraction analysis with the numbering of the glucose units is represented in Fig. 4. Bond length and bond angles observed for the difunctionalized β-CD molecule are to be considered unexceptional. A selection of torsion angles which define the orientation of the branched chains with respect to the glucose units and the primary hydroxy groups is listed in Table 2a. All glucose residues have the usual $^4C_1$ chair conformation, as derived by the Cremer and Pople puckering parameters of the glucose units.[46] The total puckering amplitudes Q for all residues (in the range 0.52–0.58 Å) are all slightly lower than the corresponding value found for an ideal cyclohexane chair conformation (0.63 Å), whereas the parameters which measure the magnitude of ring distortion are in the range 1.3–11.0°. The maximum distortions were found for the functionalized glucose units G1 and G2 (9.0° and 11.0°, respectively).

The macrocycle structure maintains an approximate seven-fold symmetry with small differences with respect to those observed for the hydrated unfunctionalized or methylated β-CDs.[5,47,48] The glucosidic O(4) atoms form a heptagon with radius and side length in the range 4.79–5.28 Å and 4.28–4.47 Å, respectively (Table 3). The angle between the three O(4) atoms of consecutive glucose units [O(4)n − 1–O(4)n–

§ COSY: homonuclear chemical-shift-correlation spectroscopy; TOCSY: total correlation spectroscopy; HSQC: heteronuclear proton-detected single-quantum coherence spectroscopy; HSQCTOCSY: combination of HSQC and TOCSY.

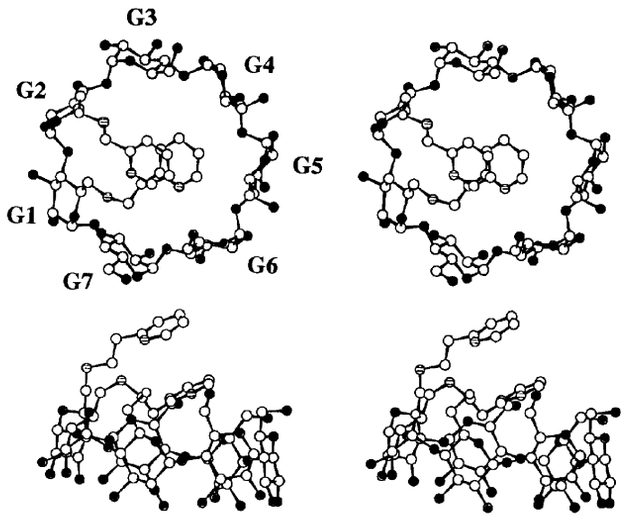

Fig. 4 Stereo view of the $6^{I},6^{II}$-dideoxy-$6^{I},6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose molecular model.

O(4)n + 1] is on average 128.3° (in the range 124.8–131.2°). The O(4) atoms are nearly coplanar with a maximum deviation from the least-squares plane passing through them of 0.26 Å. The tilt angles[49] of glucose residues, defined as the angle made by the O(4)-atoms plane and the plane formed by O(4)n + 1, C(1)n, C(4)n, and O(4)n of each glucose residue, are in the range 3.3–22.8° (Table 3). Hydroxy groups of neighbouring glucose residues are linked by intramolecular H-bonds, which stabilize the round shape of the β-CD ring: O(2)n–O(3)n − 1 H-bonds lie in the range 2.68–3.01 Å. It is interesting to note that a strong hydrogen bond (2.68 Å) is observed in ABAEPY between the hydroxy groups of the functionalized glucose rings, contrary to what has been observed in the structure of $6^{I},6^{II}$-diamino-$6^{I},6^{II}$-dideoxy-β-cyclodextrin,[35,36] in which a slightly weaker hydrogen bond was present (3.09 Å).

The primary hydroxy groups of the G3, G4, G5 and G7 glucose units assume a *gauche*$^{+}$–*gauche*$^{-}$ orientation [mean torsion angles C(4)–C(5)–C(6)–O(6) and O(5)–C(5)–C(6)–O(6), 52.8° and −67.7° respectively]. In contrast, the G6 glucose unit with a primary hydroxy group, and the G1 and G2 glucose units bearing the functionalized groups, exhibit a *gauche*$^{+}$–*trans* orientation [mean torsion angles C(4)–C(5)–C(6)–O(6) or C(4)–C(5)–C(6)–N(6) and O(5)–C(5)–C(6)–O(6) or O(5)–C(5)–C(6)–N(6), 61.2° and −176.3°, respectively]. On the other hand the G3 and G7 glucose units present two statistically disordered orientations of the primary hydroxy groups, one *gauche*$^{+}$–*gauche*$^{-}$, the other *gauche*$^{+}$–*trans*, respectively. Consequently, two (or four including the statistic hydroxy group of G3 and G7) hydroxy groups point outside the cavity of the macrocycle, while the two amino and one hydroxy group (or three including the statistical hydroxy group of the G3 and G7 units) are parallel to the edge of the macrocycle.

The host–guest interaction

The molecular model of the structure reveals that the two aminoethylpyridine groups linked to the G1 and G2 glucose units show a different conformation: the unit linked to the G2 glucose moiety extends away from the cavity of the β-CD, the other, linked to the G1 glucose moiety, remains near the mouth of the cavity. However, in the crystal the aminoethylpyridine group linked to G2 is deeply inserted into the cavity of an adjacent β-CD molecule translated along the c axis (Fig. 5). This motif repeats with the formation of long rows of β-CD molecules stabilized by these host–guest interactions. The conformation of the two aminoethylpyridine moieties is analytically described by the torsion angles given in Table 2b. In particular, the more relevant differences between the two

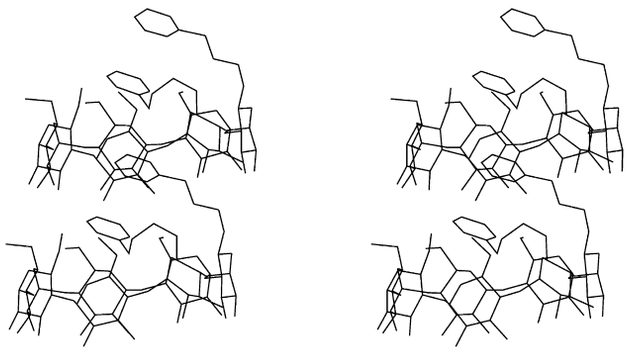

Fig. 5 Stereo view of the host–guest structure related by translation along the c axis.

moieties are found for the torsions around the C(6)n–N(6)n and N(6)n–C1 bonds (with n = 1, 2): the observed values for the C(5)n–C(6)n–N(6)n–C1 and C(6)n–N(6)n–C1–C2 torsion angles are 36.9°, 65.3° for the aminoethylpyridine group linked to G1, and 78.5°, 173.0° for the aminoethylpyridine group linked to G2, respectively. This feature allows the aminoethylpyridine group linked to G2 to be included in the host cyclodextrin cavity of a neighbouring molecule. In particular, the baricentre of the aminoethylpyridine group linked to G2 is positioned just below the O(4) plane of a translated molecule at a distance of 0.90 Å from it, with the C3B, N4B, C5B, C6B, C7B and C8B atoms at distances from this plane of 1.14, 1.71, 1.24, 0.46, 0.18 and 0.65 Å, respectively. The plane of the pyridyl ring forms an angle of 35.1° with this O(4) plane. Thus, the terminal pyridyl group linked to the G2 glucose ring is almost buried inside the β-CD macrocycle where favourable intramolecular host–guest van der Waals interactions occur with the hydrophobic cavity, which further stabilize the structure. The shortest contacts are listed in Table 4. In contrast, the other aminoethylpyridine group, linked to the G1 glucose unit, shows its pyridyl ring located on top of the β-CD cavity. The distances between the baricentre of this pyridyl group and the O(4) planes of the β-CD molecule to which it is linked and that of the β-CD molecule translated along the c axis are 3.5 Å and 2.6 Å, respectively. Furthermore, the positions of the two aromatic rings are stabilized by two strong intramolecular hydrogen bonds: the first, N–H···N (3.13 Å) between the N(6)1 amino group and the N4 atom of the pyridyl ring of the G2 unit, the other between the O(6)6 hydroxy group of the β-CD cavity entrance and the pyridyl ring N4 linked to the G1 unit ring (2.75 Å). The ability of the substituents to form strong intra- and/or intermolecular interactions is commonly observed in substituted β-CD structures and it is one of the factors involved in the stabilization of the β-CD host–guest complex.

In the structure a unique arrangement of the pyridyl rings is observed: along a row of β-CD molecules linked through the host–guest interactions along the c axis the pyridyl rings are almost parallel and nearly equidistant with each other. The distance between the baricentres of adjacent rings is 4.0 Å with an angle between the planes of the rings of 9.0°.

Molecular packing and water molecules

In Fig. 6, the mode of packing of the ABAEPY-difunctionalized β-CD molecules as viewed along the c direction is shown. Two β-CD molecules are connected along the c axis by six hydrogen bonds that involve the N(6)n and the primary hydroxy groups with the secondary hydroxy groups. This arrangement gives rise to a polymeric inclusion structure forming low rows where a single species acts both as a guest and as a host. These rows are arranged in layers of parallel molecules along the bc plane. The layers pack in an antiparallel fashion perpendicular to the a plane. In addition, the rows are held together by a complicated intermolecular hydrogen-bond network that

Table 4 Main van der Waals contacts between the pyridylethylamino moiety linked to the G2 unit and the β-CD ring of a molecule translated along the *c* axis

	β-CD	Short contacts/Å		β-CD	Short contacts/Å
C1B	C(3)2	3.96	C6B	C(3)5	4.10
C1B	O(3)2	3.57	C6B	O(4)5	3.49
C2B	O(4)1	4.00	C6B	C(4)5	4.16
C2B	C(3)2	3.55	C6B	C(5)5	4.20
C2B	O(3)2	3.70	C6B	C(3)6	4.18
N4B	C(3)7	4.06	C6B	C(5)6	4.07
C5B	C(3)6	4.19	C7B	C(3)4	3.98
C5B	O(4)6	3.76	C7B	O(4)4	3.63
C5B	C(3)7	4.04	C8B	O(4)3	3.97

The listed distances are less than 4.2 Å for C–C, and 4.0 Å for C–O and N–C.

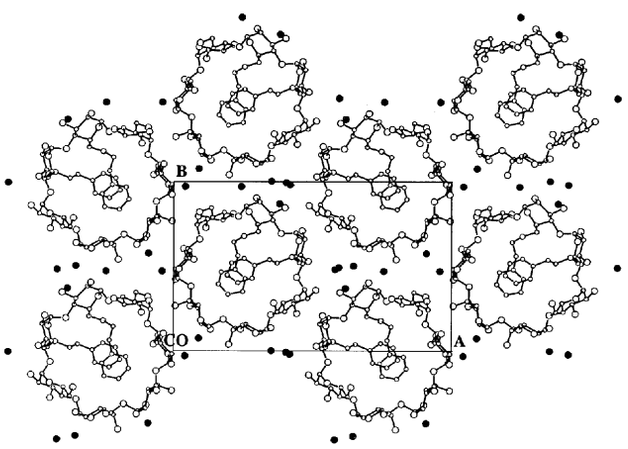

Fig. 6 Crystal packing of $6^{I},6^{II}$-dideoxy-$6^{I},6^{II}$-bis[2-(2-pyridyl)ethylamino]-β-cyclomaltoheptaose as viewed down the *c* axis.

includes direct links between primary and secondary hydroxy groups and H-bond interactions with water molecules. In particular, of the 5.5 water molecules, distributed over 7 molecular sites, present in the crystal, only the Ow_4 molecule does not link to symmetry-related molecules. All water molecules except Ow_1 and Ow_2 are involved in H-bonds with each other. In the crystal the hexagonal arrangement of β-CD molecules produces continuous hydrophilic channels filled with water molecules.

Conclusions

This study represents the first example of a branched difunctionalized β-cyclodextrin structurally characterized by single-crystal X-ray diffraction and NMR. In the solid state the macrocycle structure maintains an approximate seven-fold symmetry with small differences with respect to the structures of other β-CDs. This evidence underlines the fact that the β-CD is rigid and that only small changes occur in conformation when two consecutive primary hydroxy groups are substituted with bulky groups. In addition, the two aminoethylpyridine groups linked to contiguous glucose units show different behaviour with one extending away from the cavity of the β-CD, the other remaining near the mouth of the cavity. However, in the crystal the first aminoethylpyridine group is deeply inserted into the cavity of an adjacent β-CD molecule translated along the *c* axis giving rise to long rows of β-CD molecules stabilized by these host–guest interactions. This polymeric arrangement, already found in a crystal structure of a monosubstituted β-CD,[49] is the result of the tendency of the hydrophobic groups to be excluded from hydrophilic surroundings.

In aqueous solution an intramolecular interaction of aromatic rings with the cavity can be suggested on the basis of NMR and CD spectra. The two pyridine rings are poised perpendicularly with respect to the cavity and are included and located near the upper rim. Furthermore a stacking interaction between the two rings can be hypothesized.

The relative positions of the two moieties on the CD cavity are a very important element in the behaviour of the investigated system: the different regioisomers show very different abilities in their molecular-recognition properties[30] and in their enzyme-mimicking properties.[34] Thus investigations of the relationship between structure and complexing ability or molecular recognition ability of this class of compounds is an important step in the design of new functionalized CDs. ABAEPY could be able to complex metal ions such as copper(II) using the four nitrogen atoms. The system could be a promising model for SOD enzyme as has been found for analogous compounds.[34,50]

Acknowledgements

We gratefully acknowledge the financial support of the Ministry of Education of Italy (40% and 60%), the CNR of Italy and the Human Capital and Mobility Program of the European Community (ERBCHRXCT940484). Mr Giuseppe Perretta is also acknowledged for technical assistance.

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