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Synthesis of 6-Mono-6-deoxy-*β*-cyclodextrins Substituted with Isomeric Aminobenzoic Acids. Structural Characterization, Conformational Preferences, and Self-inclusion as Studied by NMR Spectroscopy in Aqueous Solution and by X-ray Crystallography in the Solid State

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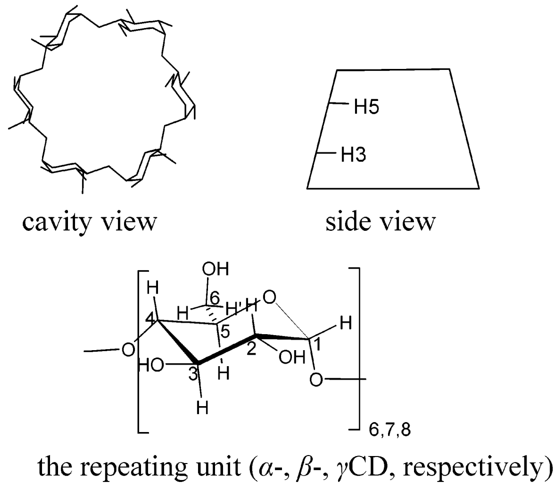
The synthesis, purification, and characterization of mono-6-modified-*β*-cyclodextrins bearing N-attached *o*-, *m*-, and *p*-aminobenzoic acids (**2**, **3**, and **4**, respectively) are presented. The structures in aqueous solution were investigated using one- and two-dimensional NMR spectroscopy. Detailed assignment of the spectra together with intramolecular NOE correlations revealed the way each of the isomeric appendages is positioned relative to the macrocyclic cavity. No self-inclusion is observed. The *o*-isomer **2** turns inward over the top of the primary side and interacts with specific protons of the substituted glucopyranose unit A and those of a neighboring unit. The *m*-isomer **3** displays two conformations, where the substituent resides above the primary side in a tilted manner and interacts either with the previous or the next unit. We propose that the carboxyl groups in both **2** and **3** are localized through H-bonding with one or two, respectively, primary hydroxyl groups of the neighboring glucopyranose units. In a similar positioning of the aromatic ring of the *p*-isomer **4**, the hydrophilic carboxyl end is fully exposed to the aqueous environment. The X-ray structure of **4** shows that the solution conformation has evolved such that in the crystalline state, the aromatic moiety is inserted through its carboxyl part inside another CD where it establishes intermolecular H-bonds with inward-turned primary OH groups. Besides this stabilization, **4** forms parallel and antiparallel supramolecular chains in the crystal that are additionally stabilized by direct H-bonds.

Introduction

Cyclodextrins (CDs) are well-known cyclic oligosaccharides[1] able to bind a large variety of molecules in their hollow structure through molecular encapsulation[2] (Scheme 1). This property has found numerous applications in various fields.[3] Chemical modifications of the natural cyclodextrins have been sought in order to increase the aqueous solubility, improve the binding properties, and enhance their chiral selectivity.[4]

The CDs, and especially *β*CD, are usually mono-6-substituted, due to the availability of the classic mono-6-tosyl-*β*CD derivative,[4b] in which the tosyl group is readily displaced by a nucleophile. This derivative can be transformed to the mono-6-deoxy-6-amino-*β*CD, which couples with amino acids via C-attachment to create an amide bond.[5] The interest in synthesizing amino acid derivatives stems from their potential use as vectorization-dedicated molecular carriers, their increased aqueous solubility, and their higher affinity toward binding of zwitterionic guests (e.g., other amino acids). The availability of the *β*-cyclodextrinyl aldehyde[6] has offered an alternative approach to derivatization since the high reactivity of the formyl group opened ways to reactions with poor nucleophiles in aqueous solution. We demon-

SCHEME 1



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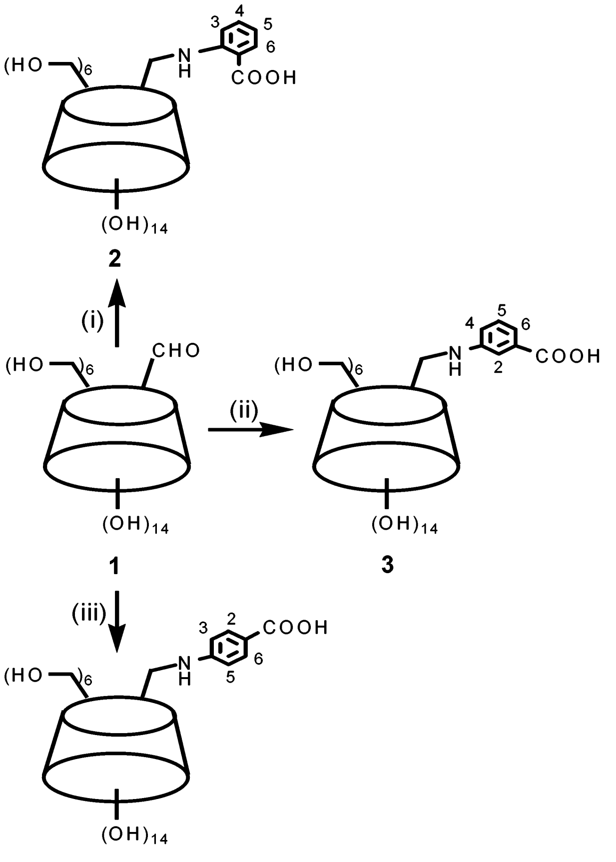
strate here the synthesis of three isomeric aromatic amino acid derivatives [mono-6-(carboxyphenylamino)-βCDs] not by creation of an amide bond but through attachment via the amine group, using the aldehyde precursor **1**. The novel derivatives **2**−**4** are βCD-linked isomers of aminobenzoic acid. Many monosubstituted βCD derivatives with commonly used similar aromatic moieties [dansyl,[5c] *p*-(dimethylamino)benzoyl,[5d] methyl red,[5g] anilino,[6b,7a−c] *m*-toluidino[7d]] that bear hydrophobic groups are invariably reported to self-include their substituents within the same CD cavity. When similar aromatic groups are attached on the βCD macrocycles through spacer molecules, the increased flexibility enhances the self-inclusion property. The present molecules hold a highly hydrophilic group, the carboxyl group, in three different positions of the phenyl substituent. The structures and preferred conformations of the three isomeric attachments were studied extensively using NMR spectroscopy in solution. Comparison between the solution and the crystalline-state structure was possible only for **4**, since derivatives **2** and **3** did not provide crystals.

Results and Discussion

The synthesis of 6-mono-6-deoxy-6-(carboxyphenylamino)-βCDs **2**, **3** and **4** was accomplished by reacting *o*-, *m*-, or *p*-aminobenzoic acid, respectively, with the known derivative mono-6-formyl-β-cyclodextrin[6] (**1**) in aqueous solution followed by in situ reduction of the Schiff base intermediate. The synthetic route is shown in Scheme 2. In the aqueous environment, the aldehyde group of **1** is partially transformed into the corresponding hemiacetal[6c] and, since both paths are equilibrium processes, the overall yields of the products **2**−**4** are moderate.

Contrary to the highly symmetrical shape of natural cyclodextrins, the monoderivatives lack symmetry elements; therefore, the complete structural identification and therefore the purity of the monoderivatives must rely on careful interpretation of NMR spectra to ensure that the compound in hand is a genuine derivative and not an inclusion complex of the monosubstituted-βCD precursor, since the latter would also give nearly identical ^{1}H NMR spectra and other spectroscopic data. The purity of the products is of major importance since the aromatic molecules themselves have a strong tendency to remain included. Actually, *o*-, *m*-, and *p*-aminobenzoic acid all form strong complexes with βCD, as we established by independent experiments and found in the literature for the *m*- and *p*-isomers.[9] This property resulted in similar R_f values of products and reactants. Contamination due to inclusion of the starting materials results in displacement of the NMR peaks, possible broadening due to moderately fast exchange, and confuses the assignments enormously; furthermore, physical properties such as the aqueous solubility of the products are altered as well. Semipreparative HPLC provided products **2**−**4** of >95% purity. Their characterization in D_2O solution was accomplished via two-dimensional (2D) homonuclear and heteronuclear NMR experiments. In the solid state, only **4** provided crystals suitable for X-ray analysis.

SCHEME 2[a]



[a] Synthetic route for derivatives **2**−**4**, pH ~6. (i) $NaBH_3CN$, H_2O, *o*-aminobenzoic acid; (ii) $NaBH_3CN$, H_2O, *m*-aminobenzoic acid; (iii) $NaBH_3CN$, $EtOH/H_2O$, *p*-aminobenzoic acid.

Structural Characterization in Solution by NMR Spectroscopy: *o*-Aminobenzoic Acid Derivative 2. The ^{1}H NMR spectrum of **2** is very well resolved in the anomeric region and shows seven doublets for each one

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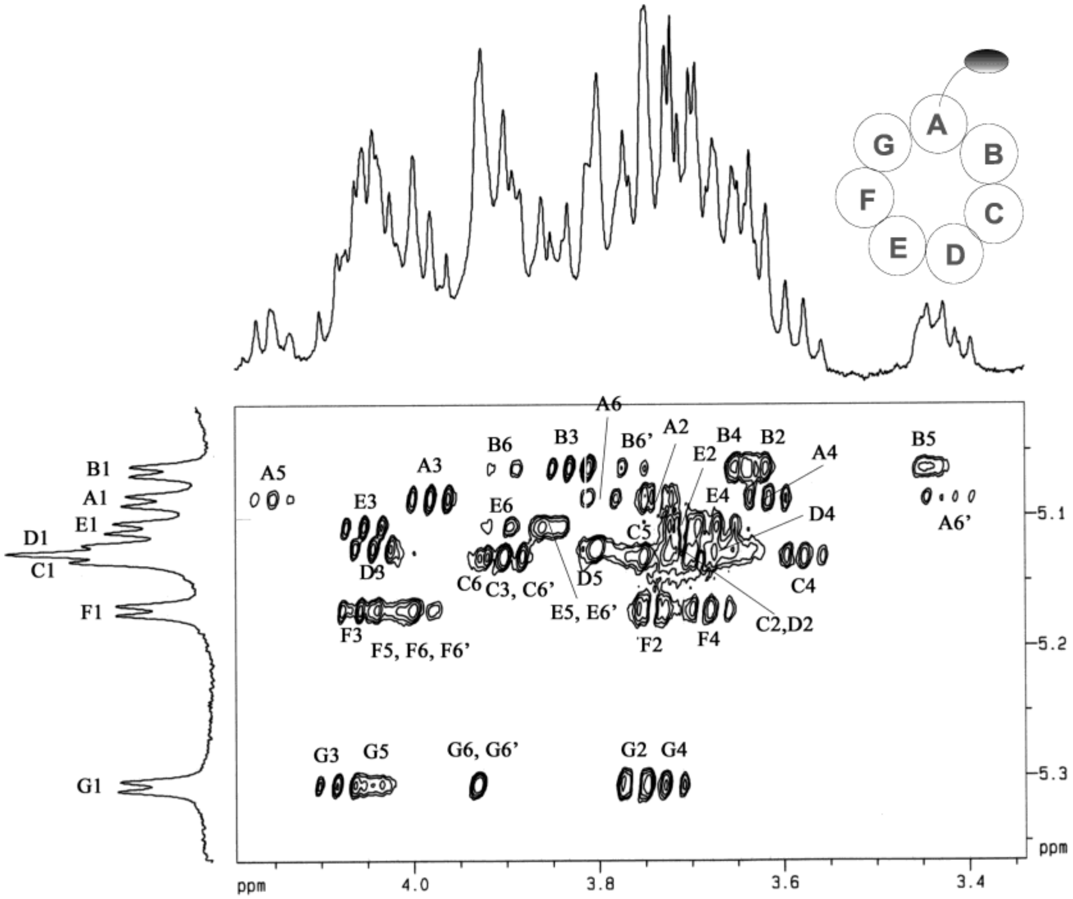

FIGURE 1. 2D TOCSY spectrum (cyclodextrin region) of derivative **2** with assignments (mixing time 160 ms, 310 K). The inset shows the labels of the consecutive glucopyranose units.

of the seven H1 atoms of the glucose units. The dispersion of the H1 signals provided the beginning of Ariadne's thread since it allowed the tracing and identification of all protons within each unit with 2D TOCSY (Figure 1), combined with COSY (Supporting Figures 1 and 4) and HSQC.[7b,10]

The latter (Figure 2) showed two protons connected to a single carbon atom appearing at 44.0 ppm, typical for a methylene carbon attached to a nitrogen atom, evidently due to H6 and H6′ (Scheme 1) of the substituent bearing unit A (Figure 1), labeled as A6 and A6′, respectively. Connectivities among successive glucose units were established from the 2D ROESY spectra by following the NOE correlations, starting from the anomeric proton A1 of unit A, with the proton B4 of unit B and so on, and cross-checking with the HSQC spectrum, which clearly defines the H4 region.

The aromatic protons (denoted as *H*) showed the anticipated pattern of *o*-benzoic acid. According to the NOE correlations observed in 2D ROESY (Figure 3 in Supporting Information), the aromatic proton *H3* correlates with protons A6 and A5, as well as with G5 of the neighboring unit G. The other aromatic protons, including *H4*, do not show any correlation with the macrocycle. Free *o*-aminobenzoic acid, as separate ROESY experiments showed, forms an inclusion complex with βCD entering the cavity with the amino and carboxyl groups toward the primary side. When covalently connected, it does not form a self-inclusion complex by entering the same or an adjacent cavity. Judging from the observed large coupling constant for A6′ (Figure 1), the C6A-NH bond seems to have assumed a (−)gauche (gauche−trans[10]) conformation with respect to unit A. The aromatic moiety, therefore, has turned inward over the primary side of the oligosaccharide. If a hydrogen bond stabilizes its conformation, it has to be between the carboxyl group and the primary hydroxyl group of the other neighboring unit, B, and models show that this is possible.

***m*-Aminobenzoic Acid Derivative 3.** Similar analysis of the TOCSY, COSY, and HSQC spectra led to complete assignment and structure elucidation of this derivative, too. The appearance, however, of the aromatic region was unusual in that the peaks were somewhat broad and overlapped (Figure 3), unlike the spectrum of free *m*-aminobenzoic acid, which is first order.

Each group of aromatic proton signals in the 2D HSQC spectrum (Figure 3) correlated with eight instead of the expected four carbon atoms, denoting the presence of two different aromatic rings. Inspection of the CD region in all available one-dimensional (1D) and 2D spectra, on the other hand, revealed only one type of substituted glucopyranose unit A. Clearly, an additional type of *m*-amino benzoic acid moiety existed in some way.

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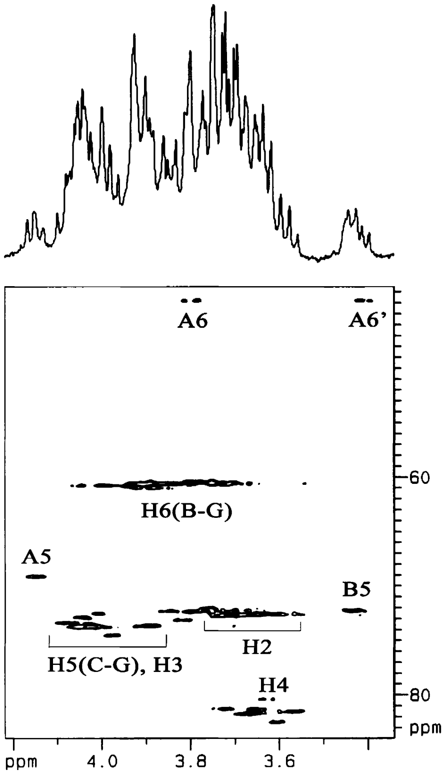


FIGURE 2. 2D HSQC spectrum (cyclodextrin region) of **2** with assignments.

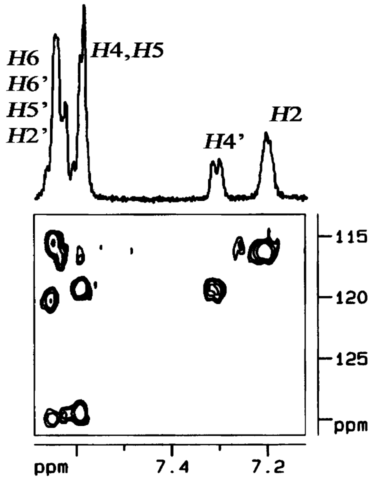


FIGURE 3. 2D HSQC spectrum (aromatic region) of **3** with assignments.

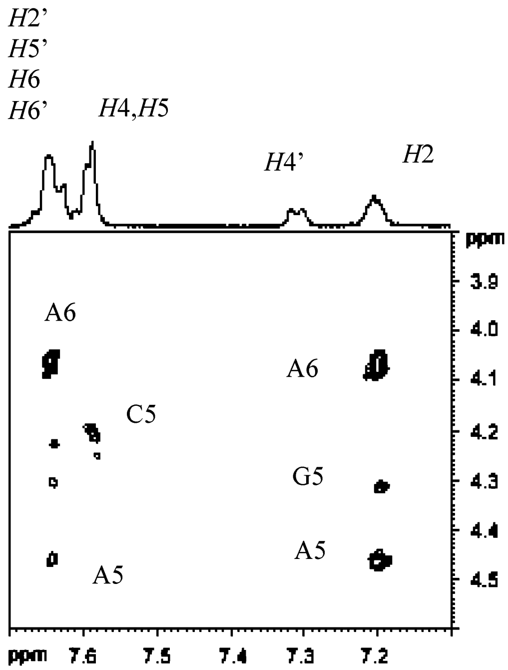


FIGURE 4. 2D ROESY spectrum of **3** with the aromatic signals attributed to two distinctly different *m*-aminobenzoic acid moieties and their respective interactions with CD protons (310 K).

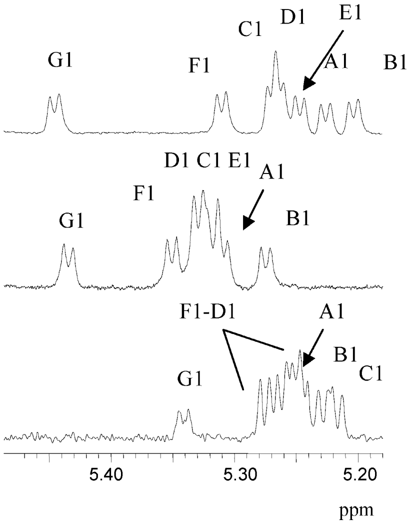


FIGURE 5. Anomeric proton (H1) region of (top to bottom): **2**−**4**.

The 2D ROESY spectrum (Figure 4) showed few and weak interactions between the aromatic protons and the CD protons. If free *m*-aminobenzoic acid was present in the cavity (not supported by the signal pattern in the aromatic region), we should observe interactions with several of the well-defined H3 protons in the CD region (4.4−4.2 ppm, Supporting Figure 6), but no such correlations were detected. We, therefore, had to consider two conformations of the appended aromatic ring. Under this light and after careful inspection of the spectra of this and of free *m*-aminobenzoic acid, and with the condition that carbons *C2* and *C5* are the most shielded and deshielded, respectively, we assigned the aromatic peaks, denoted as *H* and *H′* to the two conformations (Figure 3). The peak attributed to *H2* does not show any COSY/TOCSY interaction with the signal on its left, so the latter had to belong to the other conformation, strengthening its assignment as *H4′*. The positioning of each type of aromatic ring with respect to the macrocycle was inferred from Figure 4. We observed NOE interactions between *H2* and the hydrogen atoms A5, A6 of the same glucopyranose unit, A, and G5 of the neighboring unit G. We can attribute the cross-peak of CD proton C5 to a weak interaction with *H5*. As with **2**, models show that establishment of an H-bond of the −COOH group with the primary hydroxyls of unit G (or B) is feasible, and

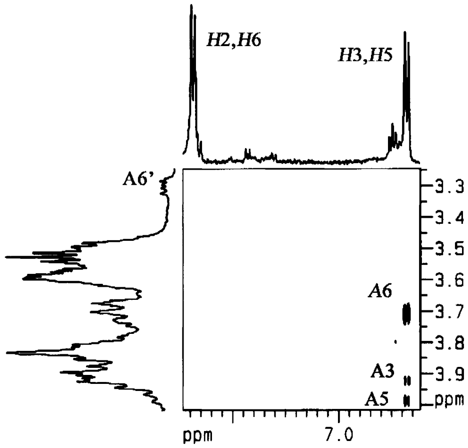


FIGURE 6. 2D ROESY spectrum of **4** and their respective interactions with glucopyranose protons (298 K).

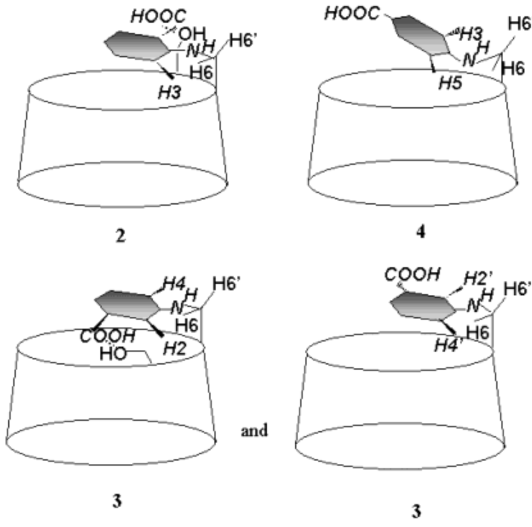
such an interaction for this conformation had to be with G. In the other conformation, the *H2′* signal overlaps with other protons, but it could be the one that gives rise to the interactions with the same CD protons as above, A5 and A6. Stabilization through H-bonding of the −COOH group with the primary hydroxyls of unit B is the logical alternative to the proposed conformation above. The remaining cross-peaks are too weak to be considered. Repeated EXCY experiments running for different mixing times (20 ms to 5 s) showed no exchange peaks between the aromatic protons *H2* and *H2′*. Temperature increase, however, to 80 °C resulted in 2-fold reduction in the intensity of H4′, indicating indeed the presence of a temperature-dependent process, as H-bonding is, although one might anticipate more dramatic events upon heating. Our data are limited and cannot be cross-checked with other methods; therefore, further elaboration on the differences of the two structures cannot be done. Last, the C6−NH−Ar bond is turned over the cavity here, too, as the large coupling constants of A6′ indicate, meaning that this proton is branching outward and rightfully does not correlate in the ROESY spectrum with aromatic protons from either conformation.

***p*-Aminobenzoic Acid Derivative 4.** This derivative was the most difficult to purify. The dispersion of the anomeric H1 signals is limited (Figure 5). The remaining CD protons are not very dispersed either, as shown by the 2D HSQC (H4, H6,6′), TOCSY (H3), and COSY (H2) spectra (Figures 8 and 9 in Supporting Information). The aromatic region is as expected (Figure 6) (the small peaks are due to an unrelated, nonseparable impurity).

The observed NOE correlations (Figure 6) are between *H3* (ortho to the amino group) and protons A3 (weak), A5, and A6 (not A6′) of unit A. No indication of full self-inclusion was observed. As previously seen, the aromatic ring is turned over the cavity, as shown by the characteristic position and coupling pattern of A6′.

We have seen that all three isomeric CDs have their aromatic part positioned over the primary side of the macrocycle, and the observed NOE interactions are between the aromatic ring protons and the primary side protons of the same or a neighboring glucopyranose unit. No self-inclusion in the derivatives takes place, although all isomeric aminobenzoic acids form inclusion complexes with βCD. We proposed that H-bonding interactions stabilize the carboxyl group in one conformation in **2** and in two conformations in **3** with the primary hydroxyl groups of nearby CDs, since in both derivatives this is structurally possible. This localization of the aromatic groups gives rise to strained macrocyclic conformations, as indicated by the significant dispersion of the protons in the CD region: the signals arising from protons H6, H6′, for example, are dispersed in a region greater than 0.5 ppm, and a few H5-type protons are even more dispersed, denoting that there exist various conformations of the primary hydroxyl groups, in both **2** and **3**. Derivative **4** is the most symmetrical, and its signals are less dispersed. The positioning of the aromatic protons *H3* and *H5* close to the inner protons of A keeps the ring "tucked" on the top, and this may not permit the entrance of the aromatic substituent in the cavity of the same or another unit (Scheme 3). So for all derivatives, the exposed hydrophilic −COOH keeps the aromatic ring from dipping inside the cavity. Derivative **4** crystallized, and its structure was solved. In the crystalline state, the aromatic pendant stands on top of its own cavity, having the C6−N bond inward and the N−Ar bond outward, in a conformation similar to what we observed in solution while at the same time entering an adjacent macrocycle to form an interesting supramolecular structure, as shown next. This self-inclusion is in accordance with reported conformations of other hydrophilic CD derivatives[11] demonstrated either with NMR or crystalline structures.

SCHEME 3. Proposed Structures of Derivatives 2−4 in Aqueous Solution



Solid-State Results. Despite repeated trials to obtain crystals from all three isomeric derivatives, only **4** crystallized, and its structure was determined. ORTEP diagrams[12] and the numbering scheme for the βCD molecule and the substituent group are given in Figure 7; C*mn* and O*mn* denote the *m*th atom within the Nth (N being A to G) glucosidic residue of βCD. The substituent group of each macrocycle is enclosed into the cavity

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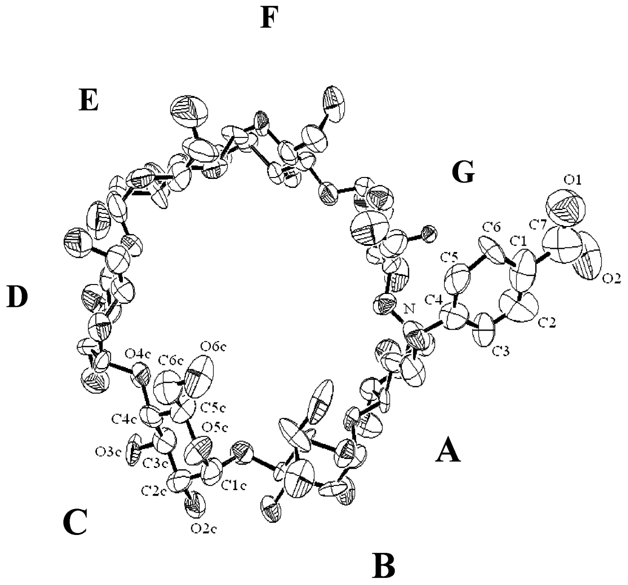

FIGURE 7. ORTEP diagram (50% probability) for the 6-mono-6-deoxy-(*p*-carboxyphenylamino)-βCD (**4**) showing the numbering scheme.

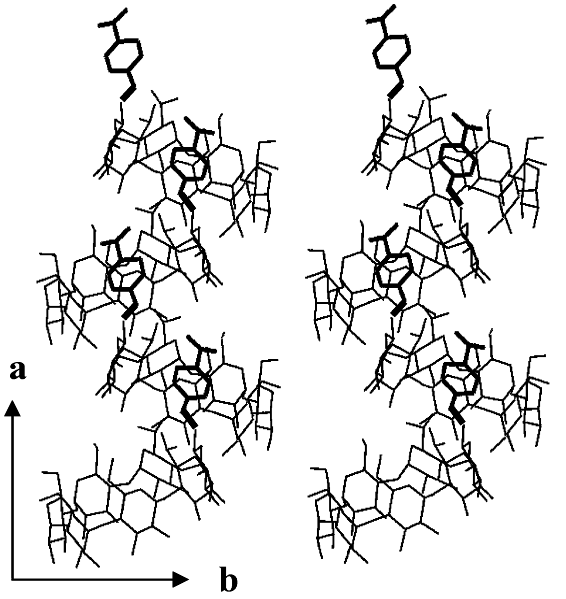

FIGURE 8. Parallel supramolecular polymeric chains of **4** along the 2-fold screw axis **a**.

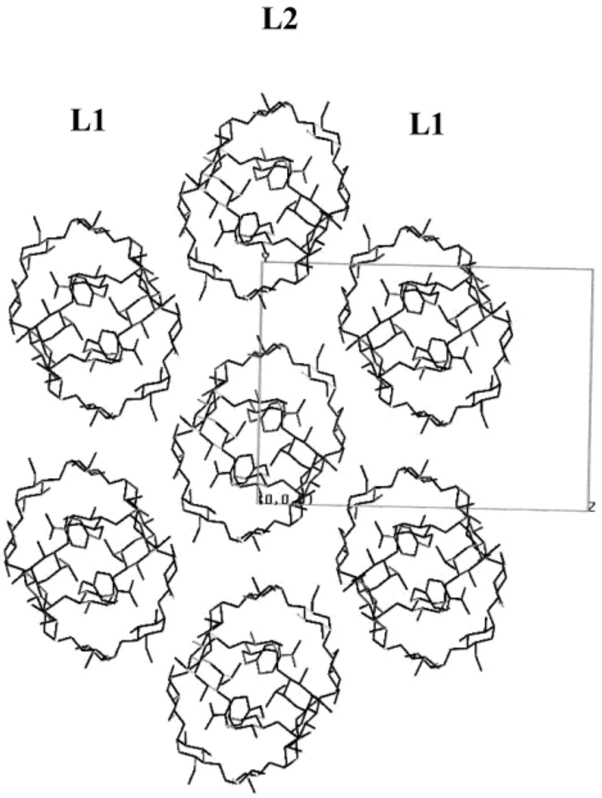

FIGURE 9. Cross-section in the **bc** plane that displays layers L1 and L2 in **4**.

of the adjacent cyclodextrin, related to the first by a 2-fold screw axis parallel to the **a** axis, thus forming infinite one-dimensional supramolecular polymers. Each cyclodextrin is inclined by 68.8° with respect to the **a** axis, and as a result, these chains are arranged in a helical fashion (Figure 8) along this axis. The crystal symmetry generates layers of supramolecular chains parallel to the **ab** crystal plane (Figure 9). Within the individual layers (L1, L2), the CD molecules have the same direction (as in Figure 8), whereas CD molecules belonging to neighboring layers are antiparallel.

The βCD macrocycle has the regular shape of βCD inclusion complexes[13] exhibiting near 7-fold symmetry as indicated by the distances between the glucosidic oxygen atoms O4*n* (range 4.30−4.42Å), their angles which do not differ significantly from the angle of the regular heptagon, as well as their deviation from the optimum plane through the O4 atoms (mean deviation 0.05 Å). The conformation of the βCD macrocycle is stabilized by the usual intramolecular hydrogen bonds between the O3*n* and O2($n + 1$) atoms of neighboring glucosidic units[13] (range 2.70−3.06Å). The glucose units tilt slightly toward the cavity. The substituted glucose A and glucose D exhibit less tilting, standing almost perpendicular to the O4 optimum plane. The conformations of the primary hydroxyl groups vary. Those of residues B and G, adjacent to the substituted glucose, are disordered, showing both the (−)-gauche (outward pointing) and (+)-gauche (inward pointing) conformations. Inward-pointing conformations (+)-gauche also have the primary hydroxyl group of residue C, which H-bonds to one carboxylic oxygen atom and the C6A−N bond in the substituted glucose A. The rest of the primary hydroxyl groups have the outward-pointing (−)-gauche conformation.

The interactions between the enclosed substituent group and the cavity resemble classical host−guest complexes. The *p*-carboxyphenylamino group of each βCD penetrates the adjacent cyclodextrin entering from its secondary side and brings the end-carboxyl group at the primary side of the adjacent βCD. The oxygen atoms O1 and O2 of the carboxyl group form H-bonds with the inward-pointing primary hydroxyl groups O6C and O6aB (Table 1, section a), respectively. Moreover, five water molecules (three have occupation factors close to 1) are

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TABLE 1. Intermolecular Distances in Derivative 4 (Atoms of an Adjacent Macrocycle Are Primed)

a. primary hydroxyl groups within H-bonding distance to the substituent's carboxyl group.

$O_{guest}\cdots O_{host}$	distance $On\cdots OmN$ (Å)	angle $C7{-}On\cdots OmN$ (deg)	angle $On\cdots OmN{-}CmN$ (deg)
O2···O6C^{iv}	3.15	101.1	93.7
O1···O6aB^{iv}	2.72	158.7	101.4

b. water molecules within H-bonding distance to substituent's carboxylic group[a]

$O_{guest}\cdots O_{water}$	distance $On\cdots O_{water}$ (Å)	angle $C7{-}On\cdots O_{water}$ (deg)
O1···OW2A_2^{v}	2.64	110.5
O2···OW6C_1^{vii}	2.21	100.0
O2···OWO2_1^{v}	2.52	111.6
O2···OWO2_2^{vi}	2.71	127.1
O2···OW2A_1^{v}	3.08	97.3

c. direct hydrogen bonds between adjacent cyclodextrins (atoms of an adjacent macrocycle are primed)

$OmN\cdots Om'N'$	distance (Å) $OmN\cdots Om'N'$	angle (deg) $CmN{-}OmN\cdots Om'N'$	angle (deg) $OmN\cdots Om'N'{-}Cm'N'$
O6aB···O2G^{iv}	2.95	121.4	123.0
O6F···O3B^{iv}	2.86	108.4	124.0
O6D··· O2C^{iii}	2.63	121.3	106.2

[a] (i) x, y, z; (ii) $-x + 1/2, -y, z + 1/2$; (iii) $-x, y + 1/2, -z + 1/2$; (iv) $x + 1/2, -y + 1/2, -z$; (v) $x + 1/2, -y + 1/2, z$; (vi) $-x + 3/2, -y, z - 1/2$; (vii) $x, y, z - 1$.

within H-bonding distance to the carboxylic oxygen atoms (Table 1, section b) and can further stabilize the system. The inclusion into the cavity and the H-bonding provide stabilization to the substituent group, confirmed by the relatively low equivalent displacement parameters (0.08−0.17 Å^2) of its atoms, which are comparable to those of the CD macrocycle. The phenyl plane forms an angle of 53.7(8)° with the macrocycle's axis, resulting in complete occupancy of the cavity. The conformation of the appended group results in an unusual close contact of 2.05 Å between hydrogen atom A6′ (calculated position) of the primary methylene bearing the substituent and the hydrogen atom of ortho carbon *C3* of the phenyl ring. This close contact has been also observed in the solution structure of **4**, as analyzed above.

Besides the stabilization offered to the supramolecular chains by the mutual inclusion and host−guest H-bonding, there is additional stabilization in the crystal by direct H-bonds between the macrocycles as indicated in Table 1, section c. Along the chains, two H-bonds are formed between the hydroxyls O6aB and O6F of one macrocycle and O2G and O3B of the adjacent one, respectively. A third direct bond along the **b** axis is observed between hydroxyls O6D of one chain and O2C of an adjacent parallel chain. Finally, the lattice is further stabilized by the H-bonding of water molecules that surround the macrocycles. There are 19 water molecules in the asymmetric unit distributed over 40 sites around the *β*CD derivative: 28 of them (the majority of high occupation factors) are within H-bonding distance from oxygen atoms of the hydroxylic groups and 5 within H-bonding distance from the carboxyl group (Table 1, section b), and 10 (mostly of low occupation factor) H-bond among themselves.

Conclusions

We have found that the aqueous solution structures of the isomeric CD derivatives **2**−**4** share some common features. The aromatic ring attached to glucopyranose unit A is turned over the primary top and interacts only with primary protons of unit A and its immediate neighbors. Two conformations of the aromatic ring are observed for **3**, but one is observed for **2** and **4**. There is macrocyclic strain in all of the derivatives, and various conformations of the primary hydroxyl groups exist; however, this is true more so in derivatives **2** and **3** than in **4**. There is no self-inclusion in the same or another macrocycle observed, and we have attributed that to the hydrophilicity of the carboxyl group, which H-bonds with primary hydroxyls (inferred from the existence of distinct conformations) and/or water molecules. In the condensed state, as is the crystal, the substituent group in **4** is inserted into an adjacent *β*-cyclodextrin ring from the secondary hydroxyl side, forming an inclusion complex. Intermolecular donor−acceptor interactions and H-bonding are responsible for the association. Due to the mutual inclusion, the observed pattern can be paralleled to a one-dimensional supramolecular polymer in which the molecules are arranged along the crystallographic 2-fold screw axis in a helical fashion. The difference in the solution and solid-state structure of **4** could be explained by the fact that self-inclusion in solution should be in equilibrium with the free structure and that, if the former becomes significant, it crystallizes out without really becoming noticeable in the dilute solution.

Experimental Section

General comments are presented in Supporting Information.

Mono-6-deoxy-6-formyl-*β*-cyclodextrin (1).[6a] Mono-6-deoxy-6-tosyl-*β*-cyclodextrin (3.0 g, 0.0023 mol) was dissolved in dry DMSO (30 mL); collidine (3 mL) was added, and the yellow solution was heated to 135° C for 3 h. The brown mixture was then added dropwise to acetone (200 mL), and the resulting precipitate was filtered off. The solid was redissolved in water (20 mL) with heating, and the solution was added dropwise to ethanol (200 mL) to afford product **3** as a white solid (2 g, 76%): R_f 0.32 (solvent system A); ESI-MS *m/z* found 1254.5 for [M + collidine]$^+$ (100%), calcd for [$C_{42}H_{68}O_{35} + C_8H_{11}N$] 1254.2; ^{1}H NMR ($D_2O$) 9.20 (s, −C*H*O), 5.20−4.90 (H1), 4.10−3.70 (m, H3, H5, H6,6′), 3.75−3.40 (H2, H4); ^{13}C (CD_3SOCD_3) δ 198.10 (−CHO), 101.76 (C1), 73.02 (C3), 72.43, 72.03 (C2, C5), 59.84 (C6), in accordance with literature data.[6]

Mono-6-deoxy-6-(*o*-carboxyphenylamino)*β*-cyclodextrin (2). Mono-6-deoxy-6-formyl-*β*-cyclodextrin (**1**) (0.1013 g, 0.089 mmol) was dissolved in H_2O (3 mL). *o*-Aminobenzoic acid (0.1516 g, 1.1 mmol, 12.33 equiv) and $NaBH_3CN$ (0.030 g, 0.53 mmol, 6 equiv) were added to the solution, and the pH was adjusted to ~6 with dilute aqueous NaOH. The solution was stirred at ambient temperature for 7 days. The pH was monitored, and a small portion of $NaBH_3CN$ was added daily. The mixture was added to acetone (32 mL) dropwise, and the resulting white precipitate was filtered off and rinsed with diethyl ether to yield a white solid (90 mg). Purification was accomplished utilizing preparative thin-layer chromatography (silica gel) eluting with solvent system B. The white product was recovered with a mixture of CH_3CN/H_2O 3:1, v/v, $R_f = 0.1$ (solvent system B). Yield 56%. A portion was purified by HPLC (yield 58%) and gave the following data: ESI-MS *m*/*q* 1254.2 (100%, M^+), calcd for $C_{49}H_{75}O_{36}N$ 1254.1; exact mass (MALDI/ FT-MS) *m*/*q* 1276.4027; calcd for $C_{49}H_{75}O_{36}N + Na$ 1276.3961; ^1H NMR (D_2O) δ 7.90 (d, $J = 7.6$ Hz, *H6*), 7.34 (m, *H4*), 6.70 (d, $J = 7.6$ Hz, *H3*), 6.60 (t, $J = 7.6$ Hz, $J = 3.6$ Hz, *H5*), 5.25−4.95 (m, H1), 4.15−3.30 (CD region, assigned in Figures 1 and 2).

Mono-6-deoxy-6-(*m*-carboxyphenylamino)βCD (3). Mono-6-deoxy-6-formyl-*β*-cyclodextrin (**1**) (0.100 g, 0.089 mmol) was dissolved in H_2O (10 mL). *m*-Aminobenzoic acid (0.1507 g, 1.1 mmol, 12.3 equiv) and $NaBH_3CN$ (0.0157 g, 0.27 mmol, 3 equiv) were added to the solution, and the pH of the solution was adjusted to ~6 with NaOH. The solution was stirred at room temperature for 7 days. The pH was monitored, and a small portion of $NaBH_3CN$ was added daily. The mixture was added dropwise to acetone (20 mL), and the resulting white precipitate was filtered off and rinsed with Et_2O to yield a white solid (98 mg). Purification was accomplished utilizing preparative thin-layer chromatography eluting with solvent system B. The white product was recovered with a mixture of CH_3CN/H_2O 3/1, v/v, $R_f = 0.1$ (solvent system B). Yield 67%. A portion was purified by HPLC (yield 39%) and gave the following data: ESI-MS *m*/*e* 1254.5 (100%, M^+), calcd for $C_{49}H_{75}O_{36}N$ 1254.1; exact mass (MALDI/ FT-MS) found 1276.3920 ($[M + Na]^+$, 100%), calcd for $[C_{49}H_{75}O_{36}N + Na] +$ 1276.3961; ^1H NMR (500 MHz, D_2O) δ 7.40−6.70 (aromatic, see assignments in Figure 3), 5.20−4.90 (m, H1) and 4.10−3.70 (assignment as shown in Figures 6 and 7 in Supporting Information).

Mono-6-deoxy-6-(*p*-carboxyphenylamino)βCD (4). Mono-6-deoxy-6-formyl-*β*-cyclodextrin **(1)** (0.0780 g, 0.0689 mmol) was dissolved in H_2O (10 mL). *p*-Aminobenzoic acid (0.1200 g, 0.868 mmol, 12.6 equiv) and $NaBH_3CN$ (0.030 g, 0.53 mmol, 8 equiv) were added to the solution, and the pH of the solution was adjusted to ~6 with NaOH. The solution was stirred at room temperature for 7 days. The pH was monitored, and a small portion of $NaBH_3CN$ was added daily. The mixture was added dropwise to acetone (20 mL), and the resulting white precipitate was filtered off and rinsed with Et_2O to yield a brown solid (60 mg). Purification was carried out on preparative thin-layer chromatography (silica gel) plates eluting with solvent system B. The white product was recovered with a mixture of CH_3CN/H_2O 3/1, v/v, $R_f = 0.1$ (solvent system B). Yield 38%. A portion was purified by HPLC (yield 35%) and gave the following data: ESI-MS *m*/*e* 1276.4 (30%, $[M + Na]^+$), calcd for $C_{49}H_{75}O_{36}N + Na$ 1277.1; exact mass (MALDI/ FT-MS) found 1276.3941 ($[M + Na]^+$, 100%), calcd for $[C_{49}H_{75}O_{36}N + Na] +$ 1276.3961; ^1H NMR (D_2O) δ 7.60 (d, $J = 7.6$ Hz *H2,6*), 6.60 (d, $J = 7.6$ Hz, *H3,5*), 5.10−4.90 (H1), 4.10−3.30 (CD region, assigned in Figures 8 and 9 in Supporting Information).

Crystal Structure Determination. The substance was dissolved in an aqueous solution and then heated to 60 °C. Colorless crystals were formed after the solution was allowed to cool slowly over a period of 5 days. The chosen single crystal was sealed into a glass capillary with mother liquor. Diffraction data were collected at room temperature on a Syntex diffractometer, with graphite monochromated Cu Kα radiation ($\lambda = 1.5418$ Å) generated by a Rigaku rotating anode. The unit cell is orthorhombic, space group $P2_12_12_1$, $a = 13.588(6)$ Å, $b = 19.172(3)$ Å, $c = 28.187(5)$ Å, $V = 7343(4)$ Å^3. Intensities for 4225 unique reflections were collected by the θ-2θ scan method. The data were corrected for Lorentz and polarization effects. The solution of the structure was obtained using the coordinates of the skeleton atoms of the 6-mono-6-deoxy-6-(1-propylamino)-*β*-cyclodextrin isomorphous structure.[14] The rest of the atoms and the cocrystallized water molecules were found by subsequent difference Fourier maps. The structure was refined by full-matrix least-squares refinement based on F^2 up to an $R_1 = 0.1071$, $wR_2 = 0.2356$, GOF = 1.128 for $F_o > 4\sigma(F_o)$ using the program SHELX97.[15] Anisotropic refinement was used only for atoms exhibiting high-temperature factors due to the limited number of data. Hydrogen atoms were added at calculated positions to the carbon and oxygen atoms, except of the disorder ones, having $U_{eq} = 1.3U'_{eq}$ of the corresponding atom. The final difference Fourier map showed residual density of $\rho_{max} = 0.44$ e/Å^3 and $\rho_{min} = -0.49$ e/Å^3.

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Supporting Information Available: NMR spectroscopy, X-ray analysis, and general experimental comments. This material is available free of charge via the Internet at http://pubs.acs.org.

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