**Synthesis of 6-Mono(carboxyphenylamino)-6-deoxy- β -cyclodextrins. Structural
Characterization, Conformational Preferences and Self-inclusion, as Studied by NMR
Spectroscopy in Aqueous Solution, and by x-Ray Crystallography.**

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A. NMR SPECTROSCOPY

Supporting Figure 1. 2D cosy spectrum of 6-mono-6-deoxy-6-(*o*-carboxyphenylamino)- β -cyclodextrin **2**, that shows the H1-H2 coupling. A-G are the consecutive glucopyranose units; Unit A bears the *o*-aminobenzoic acid substituent (D₂O, 500 MHz, 310 K).

Supporting Figure 2. 2D roesy spectrum of 6-mono-6-deoxy-6-(*o*-carboxylphenylamino)-β-cyclodextrin **2** (CD region), that shows the connectivities of the glucopyranose units A – G via detection of noe between H1 of one unit and H4 of the next; Unit A bears the *o*-amino-benzoic acid substituent (D₂O, 500 MHz, 310 K).

Supporting Figure 3. 2D roesy spectrum of 6-mono-6-deoxy-(o-carboxyphenylamino)- β -cyclodextrin **2** (aromatic region), that shows the interactions of the aromatic protons of the substituent with glycopyranose protons; Unit A bears the o-amino-benzoic acid substituent (D_2O , 500 MHz, 310 K).

Supporting Figure 4. 2D cosy 90 spectrum of 6-mono-6-deoxy-6-(*o*-carboxyphenylamino)- β -cyclodextrin **2**, that shows 2J and 3J connectivities of protons within each glucopyranose unit A – G; Unit A bears the *o*-aminobenzoic acid substituent (D₂O, 500 MHz, 310 K).

Supporting Figure 5. 2D hsqc spectrum of 6-mono-6-deoxy-6-(*m*-carboxyphenylamino)- β -cyclodextrin **3**, that shows primarily the connectivities of two different H6 and H6' protons of the glucopyranose unit A bearing the *m*-aminobenzoic acid substituent (D₂O, 500 MHz, 310 K), the dispersion of the remaining H6, H6' and of the H4.

Supporting Figure 6. 2D tocsy spectrum of 6-mono-6-deoxy-6-(*m*-carboxyphenylamino)- β -cyclodextrin **3**, that shows the connectivities of all protons within each glucopyranose unit starting from the H1 of each unit (D₂O, 500 MHz, 310 K).

Supporting Figure 7. 2D cosy spectrum of 6-mono-6-deoxy-6-(*m*-carboxyphenylamino)- β -cyclodextrin **3**, that shows 2J and 3J couplings of protons within each glucopyranose unit A – G (D₂O, 500 MHz, 310 K).

Supporting Figure 8. 2D tocsy spectrum of 6-mono-6-deoxy-6-(*p*-carboxyphenylamino)- β -cyclodextrin **4**, that shows the connectivities of protons within each glucopyranose unit. The assignment of units A, B and G is certain, the others are not exact due to overlapping (D_2O , 500 MHz, 298 K).

Supporting Figure 9. 2D cosy spectrum of 6-mono-6-deoxy-6-(*p*-carboxyphenylamino)-6-deoxy- β -cyclodextrin **4**, that shows 2J and 3J couplings of protons within each unit (left) and H1-H2 (right). There is extensive overlapping of the signals of same type protons of most of the units making complete assignment within each unit impossible.

B. X-Ray Analysis

Supplementary Material

Supporting Table 1. Crystal data and structure refinement of 6-mono-6-deoxy 6-(p-carboxyphenylamino)- β -cyclodextrin **4**

Molecular formula	C ₄₉ H ₇₅ N O ₃₆ · 19.0H ₂ O
Formula weight	1254.10
Temperature	293 K
Radiation/Wavelength	CuK α /1.5418 Å
Space group	P 2 ₁ 2 ₁ 2 ₁
a	a = 13.588(6)Å
b	b = 19.172(3)Å
c	c = 28.187(5)Å
Volume / Z	7343(4) Å ³ / 4
Density (calculated)	1.134 Mg/m ³
2 θ range for data collection	5.58° - 100.16°
Index ranges	0 ≤ h ≤ 13, 0 ≤ k ≤ 19, 0 ≤ l ≤ 28
Reflections collected / independent	4225 / 4225
Solution method	Isomorphous molecular replacement
Refinement method	Full matrix least squares on F ²
Data/restraints/parameters	4225 / 19 / 1002
Goodness of fit on F ²	1.128
Final R indices [F _o > 4σ(F _o)]	R1 = 0.1071, wR2 = 0.2356
R indices (all data)	R1 = 0.2156, wR2 = 0.3065
Largest diff. peak and hole	0.44 and -0.49 e Å ⁻³

Supporting Table 2. Characteristics of the β -cyclodextrin macrocycle in **4**

Residue	D ^a (Å)	ϕ ^b (°)	d ^c (Å)	α ^d (°)	D ₃ ^e (Å)	Torsion angle(°) O5n-C5n- C6n-O6n
A	4.41(3)	127.8(5)	0.04(1)	3(1)	2.74(2)	73(3)
B	4.30(2)	126.7(5)	0.05(1)	13(1)	2.83(2)	67(4) -63(4)
C	4.39(2)	129.4(4)	-0.12(1)	10.1(7)	2.93(2)	57(3)
D	4.42(2)	129.4(5)	0.07(1)	2(1)	2.70(3)	-70(3)
E	4.37(2)	127.9(5)	0.00(1)	13(1)	2.83(2)	-73(4)
F	4.37(3)	125.7(5)	0.01(1)	13(1)	3.06(3)	-69(3)
G	4.39(3)	132.4(5)	-0.06(1)	24(2)	2.83(3)	75(4) -62(5)

^a Distances between adjacent O4 atoms^b Angles between adjacent O4 atoms^c Deviations (Å) from the least-squares optimum plane of the seven O4 atoms.^d Dihedral angles between the O4 plane and optimum planes through O4(previous glucose unit) and C1N, C4N, O4N.^e Intramolecular hydrogen-bond distances between atoms O3N...O2(next glucose unit).

Supporting Table 3. Hydrogen bonds between β CD hydroxyl groups, Om(a or b)N and water molecules of derivative **4**.

H-Bond	Distance (Å)	Angle (°)	H-Bond	Distance (Å)	Angle (°)
O2A ... OW2A_1	2.57	136.0	O2E ... OW2E_1	2.83	110.3
... OW2A_2	2.89	118.1	... OW3E_2	2.89	116.8
			... OW2E_2	2.96	132.0
			... OW6E	3.11	101.4
O3A ... OW3A_1	2.45	129.4	O3E ... OW3E_1	2.75	138.6
... OW3A_2	2.99	125.4	... OW6E	2.92	122.8
... OW2E_2	3.13	114.3	... OW3E_2	2.97	118.4
O2B ... OW2B_1	2.59	102.1	O6E ... OW6E_1	2.98	127.4
... OW2B_2	2.60	97.4	... OW3E_1	2.79	101.7
			... OW2E_1	2.98	135.0
			... OW6E_2	3.09	126.3
O6aB ... OW3G_2	3.08	104.1	O2F ... OW2F_1	2.61	115.6
... OW3G_1	2.78	106.6	... OW2F_2	3.01	93.6
O6bB ... OW2D	3.09	135.4	O3F ... OW3F_1	2.77	132.0
			... OW3F_2	2.39	161.7
O3C ... OW3C	2.59	144.3	O6F ... OW6F	2.93	93.6
... OW6D	2.84	140.4			
O6C ... OW6C_1	2.26	121.3	O2G ... OW3G_1	2.29	107.1
... OW6C_2	2.74	121.6	... OW2G_1	2.72	108.0
			... OW2G_2	2.95	140.4
O2D ... OW2D	2.86	110.9	O3G ... OW3G_1	2.55	102.5
			... OW3G_2	2.68	128.3
O3D ... OW3D_1	2.68	122.6	O6aG...OW3A_2	2.87	145.5
... OW3D_2	3.03	129.9	...OW2A_2	2.97	111.8
O6D ... OW6D	2.82	136.2			

C. EXPERIMENTAL

General. The nuclear magnetic resonance (NMR) spectra were acquired at 500 MHz, at 298 or 310 K, in deuterated water (D₂O) or dimethyl sulfoxide (DMSO-d₆). The 2D spectra: cosy, tocsy (mixing time 160 ms), hsqc, hmbc, noesy, roesy (mixing time 350 ms) were acquired using the pulse sequences provided in the software's library (with water elimination when needed) employing either gradient selection, or phase cycling. Thin layer chromatography was performed on aluminum supported silica gel plates with solvent system A (*n*-butanol/ethanol/water, 5:4:3) or B (*i*- propanol/ethyl acetate/water/25% aqueous ammonia, 7:7:4:5). Cyclodextrin compounds were visualized by charring with methanol/acetic acid/sulfuric acid/*p*-anisaldehyde (200:20:10:1) or 10 % sulfuric acid in ethanol spray, whereas UV exposure revealed the aromatic substituted products. Preparative thin layer chromatography was performed on home-made glass plates, holding ~15 g silica gel on which 45-80 mg of crude product were applied. Sephadex G25 column packed in acetonitrile/water (20/80, v/v) and eluted with the same mixture did not give satisfactory purification for **3**. Finally, HPLC was ran using a semi-preparative reverse phase cyano-column 250 × 4.6 mm with methanol/water (20/80, v/v) as the mobile phase, flow rate 0.5 mL/min, loop volume 20 µl, room temperature. Detection was accomplished using a UV detector at 270 nm.