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Synthesis and conformation of dihistamine derivatives of cyclomaltoheptaose (β-cyclodextrin)

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ABSTRACT

The three isomeric dihistamine derivatives of β-cyclodextrin (cyclomaltoheptaose) have been prepared and investigated by ^{1}H- and ^{13}C-n.m.r. spectroscopy. For each isomer, neither of the imidazole rings is included in the cavity, but a reciprocal influence of the histamine chains is observed, suggesting that they have an organised structure.

INTRODUCTION

Cyclomalto-oligosaccharides (cyclodextrins, CDs) show many features that are typical of enzymes, such as specificity and formation of a complex with a substrate prior to chemical transformation, often at an enhanced rate[1]. Since CDs carry only hydroxyl groups, many attempts have been made to introduce other functional groups in order to improve their applicability as enzyme models[2–9]. Furthermore, CDs functionalised with groups that interact with metal ions can provide models for metalloenzymes[10–14] since the specific binding of metal ions is a prerequisite for their catalytic function[15,16].

In this context, we now report the synthesis of the three isomeric dihistamine derivatives of βCD, 6^{A},6^{X}-dideoxy-6^{A},6^{X}-di-[2-(4-imidazolyl)ethylamino]cyclomaltoheptaose where X = B, C, and D (Scheme 1), and the use of n.m.r. spectroscopy to investigate the interactions of the histamine groups in each isomer.

EXPERIMENTAL

βCD (Sigma) was dried *in vacuo* for 24 h at 90° by using a P_2O_5 trap. Pyridine was distilled after boiling under reflux over KOH for 10 h and over BaO for 12 h. *N,N*-Dimethylformamide was distilled under reduced pressure after boiling under reflux over CaH_2 for 10 h. Each solvent was collected over molecular sieves.

T.l.c. was carried out on silica gel plates (Merck 60 F_{254}). CD derivatives were detected with u.v. light and the anisaldehyde reagent[17], and histamine derivatives by the Pauly test[18]. Merck Lichroprep RP-8 (40–63 μm) was used for reverse-phase column

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chromatography. H.p.l.c. was performed with a Varian Model 5000 instrument using a Lichrosorb-NH_2 column (5 × 250 mm, 5 μm).

6^A,6^B-Dideoxy-6^A,6^B-di-[2-(4-imidazolyl)ethylamino]cyclomaltoheptaose (**3**, *ABHm-βCD)*. — To a solution of dry βCD (3 g) in pyridine (55 mL) at 25° was added benzene-1,3-disulfonyl chloride[19] (0.27 g) in pyridine (2.5 mL) dropwise during ~ 1 h with stirring[20]. After another 1 h, the solvent was evaporated at 30° *in vacuo*, the residue was stirred with acetone, and the resulting solid was collected by filtration and subjected to reverse-phase column (35 × 500 mm) chromatography using a linear gradient (10% → 30%) of ethanol in water (total volume, 1.5 L) to eliminate βCD and minor products. Elution with aqueous 30% ethanol (~ 900 mL) then gave the 6^A,6^B-*O*-(benzene-1,3-disulfonyl) derivative **1** (0.35 g), R_F 0.55 (PrOH–H_2O–AcOEt–NH_3, 5:3:2:1), $[\alpha]_D^{25}$ + 120° (*c* 1, *N,N*-dimethylformamide). ^{1}H-N.m.r. data (250 MHz, D_2O): δ 8.6–7.78 (m, 4 H, benzene H), 5.2–4.8 (m, 7 H, H-1 of βCD), 4.5–3.0 (m, 42 H, H-3,6,5,2,4 of βCD).

A solution of dry **1** (0.35 g) and NaI (1 g) in *N,N*-dimethylformamide (10 mL) was stirred at 90° for 5 h, then concentrated to dryness in vacuum at 30°. The residue was stirred vigorously with acetone, and the resulting solid was collected by filtration and washed with acetone to remove NaI. The product was precipitated from aqueous solution with acetone to give the 6^A,6^B-dideoxy-6^A,6^B-di-iodo derivative **2**, R_F 0.34 (PrOH–H_2O–AcOEt–NH_3, 5:3:3:1).

To a solution of dry **2** (0.3 g) in *N,N*-dimethylformamide (3 mL) under nitrogen was added histamine (0.3 g). The mixture was kept for 12 h at 60°, then concentrated *in vacuo* at 40°. The syrupy residue was stirred with acetone, and the resulting solid was collected by filtration, precipitated from aqueous solution with acetone, and purified by elution from a column (20 × 600 mm) of CM-Sephadex C-25 (NH_4^+ form) with water (400 mL), then with a linear gradient 0 → 0.35M NH_4HCO_3 (total volume, 1.1 L). The appropriate 4-mL fractions were combined and concentrated to give **3** (ABHm-βCD, 0.07 g), which was homogeneous in h.p.l.c., R_F 0.46 (PrOH–H_2O–NH_3, 5:3:1), $[\alpha]_D^{25}$ +124° (*c* 1, water). ^{1}H-N.m.r. data (600 MHz, D_2O): δ 7.61 (s, 1 H, A imidazole H-2), 7.60 (s, 1 H, B imidazole H-2), 6.87 (s, 1 H, A imidazole H-3), 6.83 (s, 1 H, B imidazole H-3), 4.87–5.05 (m, 7 H, H-1 of βCD), 4.05–3.75 (m, 24 H, H-3,5,6 of βCD), 3.77–3.62 (m, 12 H, H-2,4 of βCD), 3.47 (t, 2 H, $J_{4A,5A} = J_{4B,5B}$ = 10 Hz, H-4_A,4_B), 3.13 (d, 1 H, $J_{6aA,6bB}$ 11 Hz, H-6a^A), 3.02 (d, 1 H, $J_{6aB,6bB}$ 11 Hz, H-6a^A), 2.97–2.70 (m, 10 H, histamine chain methylene protons, H-6b^A, 6b^B.

Anal. Calc. for $C_{52}H_{84}N_6O_{33}\cdot 6H_2O$: C, 43.7; H, 6.7; N, 5.9. Found: C, 43.8; H, 6.5; N. 5.6.

6_A,6_C-Dideoxy-6_A,6_C-di-[2-(4-imidazolyl)ethylamino]cyclomaltoheptaose (**6**, *AC-Hm-βCD)*. — To a solution of dry βCD (2 g) in pyridine (50 mL) at 25° was added benzophenone-3,3′-disulfonyl chloride[21] (0.6 g) with stirring[12]. Stirring was continued for 5 h, the pyridine was evaporated, the syrupy residue was stirred with acetone, and the resulting solid was collected, washed with acetone, and precipitated from aqueous solution with acetone. The crude product was purified by reverse-phase column (35 × 500 mm) chromatography by elution with a gradient (10% → 30%) of ethanol in water

(total volume, 1.2 L) in order to remove βCD and secondary products. A gradient elution with 25%→40% of ethanol (total volume, 2 L) then gave the $6_A,6_C$-*O*-(benzophenone-3,3′-disulfonyl) derivative **4** (0.40 g), R_F 0.32 (PrOH–H_2O–AcOEt–NH_3, 5:3:3:1), $[\alpha]_D^{25}$ +87° (*c* 1, *N,N*-dimethylformamide). ^{1}H-N.m.r. data: δ 8.4–8.1 (m, 4 H, benzene H-2,6), 8.1–7.9 (m, 4 H, benzene H-4,5), 5.42 (d, 1 H, $J_{1A,2A}$ 3.3 Hz, H-1^A), 5.32 (d, 1 H, $J_{1C,2C}$ 3.4 Hz, H-1^C), 5.1–4.9 (m, 5 H, other H-1 of βCD), 4.7–3.2 (m, 42 H, H-3,6,5,2,4 of βCD).

Eluted before **4** was the $6_A,6^D$-isomer (0.08 g). ^{1}H-N.m.r. data: δ 8.4–8.1 (m, 4 H, benzene H-2,6), 8.1–7.8 (m, 4 H, benzene H-4,5), 5.37 (d, 1 H, $J_{1A,2A}$ 3.3 Hz, H-1^A), 5.21 (d, 1 H, $J_{1D,2D}$ 3.4 Hz, H-1^D), 5.1–4.9 (m, 5 H, other H-1 of βCD), 4.7–4.3 (m, 4 H, H-6 of A and D residues), 4.2–2.5 (m, 38 H, H-3,6,5,2,4 of βCD).

Treatment of **4** (0.40 g) as described[12], but with NaI instead of KI, gave the $6_A,6_C$-dideoxy-$6_A,6_C$-di-iodo derivative **5** (0.35 g), R_F 0.34 (PrOH–H_2O–AcOEt–NH_3, 5:3:3:1).

Treatment of **5** (0.30 g) with histamine as described[12] gave **6** (ACHm-βCD, 0.08 g), R_F 0.46 (PrOH–H_2O–NH_3, 5:3:1), $[\alpha]_D^{25}$ +121° (*c* 1, water). ^{1}H-N.m.r. data (600 MHz, D_2O): δ 7.63 (s, 2 H, A and C imidazole H-2), 6.79 (s, 1 H, A imidazole H-3), 6.76 (s, 1 H, C imidazole H-3), 4.89–5.00 (m, 7 H, H-1 of βCD), 4.05–3.80 (m, 24 H, H-3,5,6 of βCD), 3.80–3.65 (m, 12 H, H-2,4 of βCD), 3.42 (t, 2 H, $J_{4A,5A} = J_{4C,5C}$ = 10 Hz, H-4^A, 4^C), 3.16 (d, 2 H, $J_{6aA,\ 6bA} = J_{6aC,\ 6bC}$ = 11 Hz, H-$6a^A$, $6a^C$), 2.97–2.70 (m, 10 H, histamine chain methylene protons, H-$6b^A$, $6b^C$).

Anal. Calc. for $C_{52}H_{84}N_6O_{33}\cdot 6H_2O$: C, 43.7; H, 6.7; N, 5.9. Found: C, 43.9; H, 6.6; N, 5.5.

$6_A,6^D$-Dideoxy-$6^A,6^D$-di-[2-(4-imidazolyl)ethylamino]cyclomaltoheptaose (**9**, *ADHm-βCD*). — To a stirred solution of dry βCD (3 g) in pyridine (75 mL) was added biphenyl-4,4′-disulfonyl chloride[22,23] (0.80 g) in four portions during 1 h[23]. After a further 2 h, the pyridine was distilled off, acetone was added to the residue, and the solid (~4 g) was collected by filtration, and washed by acetone. This product was subjected to reverse-phase column (35 × 500 mm) chromatography by elution with a gradient of ethanol (10%→20%) in water (total volume, 1 L) in order to remove βCD and secondary products. A further gradient of ethanol (20%→40%; total volume, 2 L) was applied, and appropriate fractions (10 mL) were combined and concentrated to give the $6_A,6^D$-*O*-(biphenyl-4,4′-disulfonyl) derivative **7** (0.40 g), R_F 0.55, (PrOH–H_2O–AcOEt–NH_3), 5:4:3:1), $[\alpha]_D^{25}$ +82° (*c* 1, *N,N*-dimethylformamide). ^{1}H-N.m.r. data: δ 8.2–8.0 (m, 8 H, benzene H), 5.2–5.0 (m, 7 H, H-1 of βCD), 4.6–2.7 (m, 42 H, H-3,6,5,2,4 of βCD).

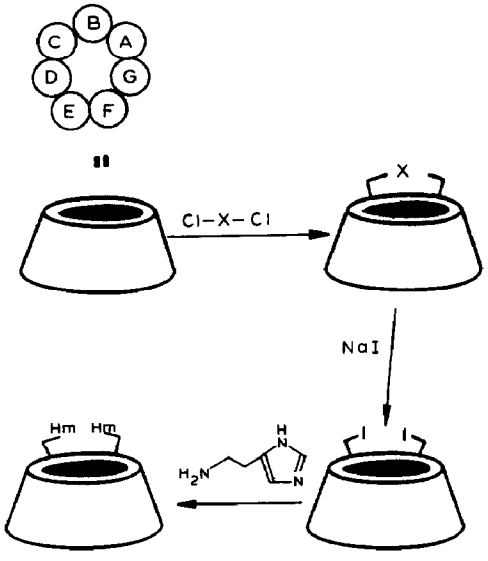
Treatment of **7** (0.40 g) with sodium iodide in *N,N*-dimethylformamide, as described for **2**, gave, after removal of insoluble biphenyl-4,4′-disulfonate salt and evaporation of the solvent, the $6_A,6^D$-dideoxy-$6_A,6^D$-di-iodo derivative **8** (0.30 g), R_F 0.34 (PrOH–H_2O–AcOEt–NH_3, 5:3:3:1).

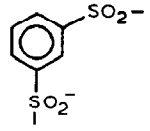
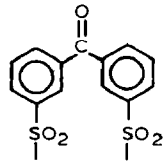
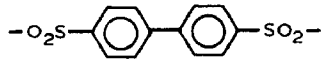
Treatment of **8** (0.30 g) with histamine, as described above, gave **9** (ADHm-βCD, 0.08 g), R_F 0.46 (PrOH–H_2O–NH_3, 5:3:1), $[\alpha]_D^{25}$ +110° (*c* 1, water). ^{1}H-N.m.r. data (600 MHz, D_2O): δ 7.64 (s, 1 H, A imidazole H-2), 7.63 (s, 1 H, D imidazole H-2), 7.09 (s, 2 H, A and D imidazole H-3), 5.12–5.02 (m, 7 H, H-1 of βCD), 4.05–3.80 (m, 24 H,

H-3,5,6 of βCD), 3.80–3.65 (m, 12 H, H-2,4 of βCD), 3.42 (t, 2 H, $J_{4A,5A} = J_{4D,5D} = 10$ Hz, H-4^A,4^D), 3.16 (d, 2 H, $J_{6a,6b}$ 11 Hz, H-6,b^A,$6b^D$), 2.97–2.70 (m, 10 H, histamine chain methylene protons, H-$6b^A$,$6b^D$).

Anal. Calc. for $C_{52}H_{84}N_6O_{33}\cdot 6H_2O$: C, 43.7; H, 6.7; N, 5.9. Found: C, 43.6; H, 6.4; N, 5.7.

N.m.r. measurements. — ^{1}H-N.m.r. spectra (600 MHz) were recorded with a Bruker AMX-600 spectrometer and ^{13}C-n.m.r. spectra (62.9 MHz) with a Bruker AC-250 spectrometer on solutions in D_2O without a reference compound. Since most of the usual reference compounds interact with the βCD cavity, the ^{1}H-n.m.r. spectra were referenced to water.



Isomer	X
A.B	
A.C	
A.D	

RESULTS AND DISCUSSION

The dihistamine derivatives of βCD were synthesised by the route shown in Scheme 1. Reaction of βCD with the appropriate disulfonyl chloride in pyridine gave the $6_A,6^X$-cyclic disulfonate which, with sodium iodide in *N,N*-dimethylformamide, gave the $6_A,6^X$-dideoxy-$6_A,6^X$-di-iodo derivative. Reaction with histamine in *N,N*-dimethylformamide then gave the dihistamine derivative.

The ^{1}H-n.m.r. spectra of ABHm-βCD (**3**), ACHm-βCD (**6**), and ADHm-βCD (**9**) at 600 MHz are shown in Figs. 1 and 2. The spectra in Fig. 1 show similarities to that[24] of

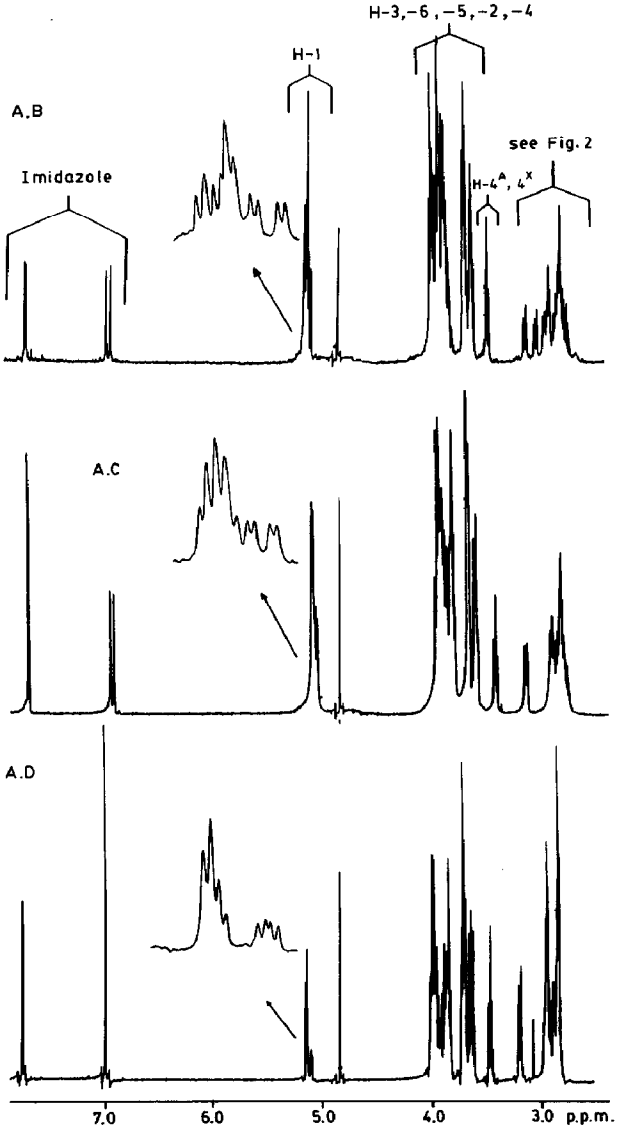


Fig. 1. ^{1}H-N.m.r. spectra (600 MHz) of ABHm-βCD (**3**), ACHm-βCD (**6**), and ADHm-βCD (**9**).

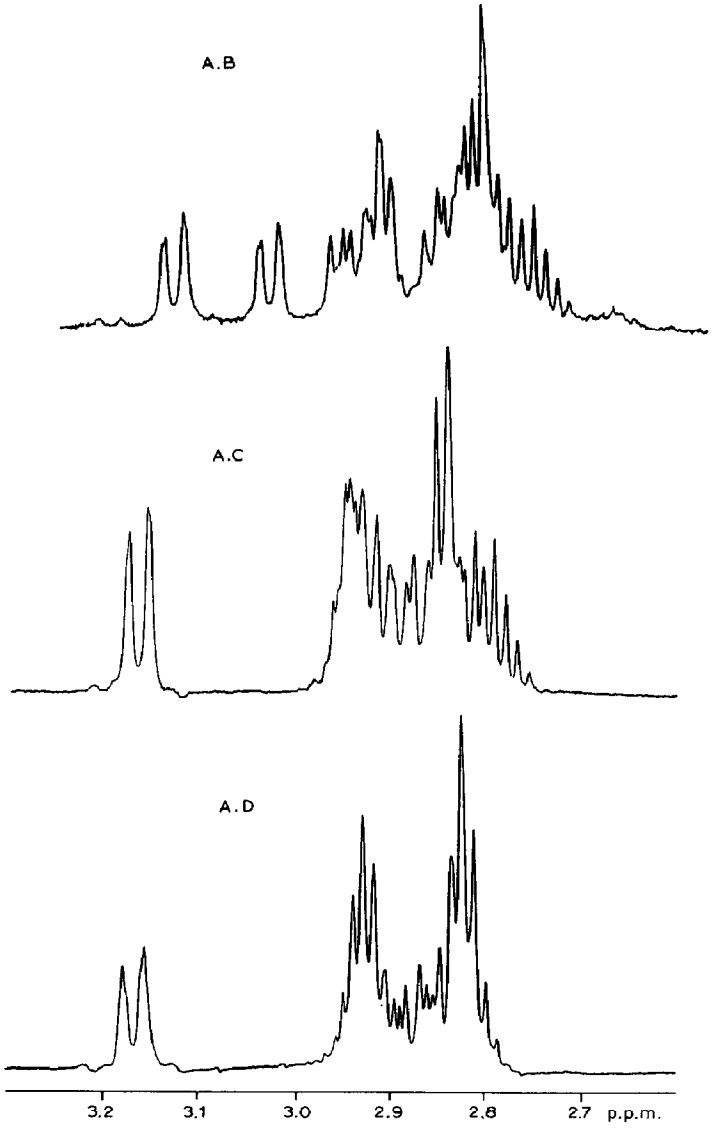


Fig. 2. ^{1}H-N.m.r. spectra (600 MHz) of ABHm-βCD (**3**), ACHm-βCD (**6**), and ADHm-βCD (**9**) in the region 3.2–2.7 p.p.m.

the monohistamine derivative of βCD which, together with the COSY ^{1}H-n.m.r. spectra, has been used to assist in the assignments to the disubstituted isomers. There are differences in the spectra in the region for aromatic protons (4 peaks for the A,B-isomer, three each for the A,C- and A,D-isomers).

In the region for anomeric protons, there are slight but significant shifts due to small through-chain effects in the substituted glucosyl residues and to small ring-current effects due to the imidazole rings.

In addition to the two usual groups of peaks observed in the ^{1}H-n.m.r. spectra of

βCDs (4.1–3.8 p.p.m. for H-3,5,6, 3.75–3.55 p.p.m. for H-2,4), peaks are present in the region 2.7–3.5 p.p.m. due to the double substitution.

The triplet in each spectrum at 3.45 p.p.m. is assigned to H-4 of the substituted glucosyl residues. This equivalence supports the through-chain origin of the upfield shift of these two resonances.

At lower frequencies (Fig. 2), the resonances for the A,B-isomer at 3.13 (d) and 3.02 (d) p.p.m. are assigned to H-6a of each substituted residue. In the A,C- and A,D-isomers, these protons are equivalent [3.15 (d) and 3.16 p.p.m. (d), respectively]. The complex multiplets at 2.7–3.0 p.p.m. are assigned to the eight histamine methylene protons, together with the H-6b of each substituted residue. As observed[24] for Hm-βCD, the resonances of the histamine methylene groups show a high multiplicity. The COSY spectra identified the multiplets for H-5, which coincide for all the isomers. Whereas, the individual multiplet for H-6a in the A,D-isomer indicated that all the H-6a are equivalent, they are not in the A,B- and A,C-isomers.

The INEPT ^{13}C-n.m.r. spectra of the A,B-, A,C-, and A,D-isomers showed strong similarities and to that of Hm-βCD. However, the small differences are significant, as illustrated by the resonances of the methylene carbons.

One peak is observed for the A,D-isomer for each kind of histamine methylene carbon and there is only a slight difference (~ 0.05 p.p.m.) in the resonances of C-6_A and C-6^D. In the A,C- and A,B-isomers, all the histamine methylene carbons, and also C-6_A and C-6^X, are not equivalent. Only for the A,D-isomer are two peaks observed for C-6 of the unsubstituted residues. The observations for the methylene carbons are paralleled by those for the methyne carbons.

Thus, the ^{13}C-n.m.r. spectra show an increased differentiation of the histamine carbons at a closer separating distance of the chains, whereas an increased differentiation of the cavity carbons is observed for a further separating distance of the chains.

The ^{1}H- and ^{13}C-n.m.r. data suggest that in the A,B-, A,C-, and A,D-isomers, there is no inclusion of the imidazole rings in the cavities. The slight effect of the histamine groups on the inward-pointing non-labile protons and the slight difference in the chemical shifts of the ^{13}C resonances of the unsubstituted residues, especially in the A,D-isomer, may reflect small ring-current effects of the two imidazole rings which also slightly affect H-1.

The high multiplicity of the ^{1}H resonances of the histamine methylene groups may reflect the occurrence of intrachain hydrogen bonds, involving the nitrogen atoms of each histamine moiety.

The partial (A,C-isomer) and complete (A,B-isomer) loss of equivalence of H-6a,6b of the substituted glucosyl residues suggests an increasing interaction of the two histamine chains as the separating distance is decreased. The different resonances of the aromatic protons provide further evidence of this effect. There are two possibilities, namely, through-space or through-chain interactions. For the latter effect, however, the cavity atoms should show a differentiation at least equal to that observed for those of the chains, which was not observed. Moreover, the ^{13}C-n.m.r. spectra show that it is in the

A,D-isomer, where the histamine groups are most remote from each other, that there is a greater differentiation of the cavity atoms.

Thus, the n.m.r. data suggest that, in the A,X-isomers **3**, **6**, and **9**, there is some organisation of the histamine groups, which, together with the presence of the cavity, makes these molecules potential two-recognition-site abiotic receptors.

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