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Synthesis and Intramolecular Inclusion Studies of Tryptophan-modified-β-cyclodextrins

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Abstract. β-cyclodextrins functionalized by D or L-tryptophan were synthesised. NMR, circular dichroism and fluorescence investigations were carried out showing the clear intramolecular inclusion of the tryptophan in the cyclodextrin cavity. The derivatives act as a fluorescent sensor which is useful for detecting organic species in solution. Furthermore, derivatives L and D show different sensitivity with regard to their interaction with a guest. The difference might be due to the disposition of the indole with respect to the cavity of the cyclodextrin, induced by the chirality of the tryptophan.

Key words: functionalized cyclodextrins, self-inclusion, spectroscopic investigations, inclusion complexes

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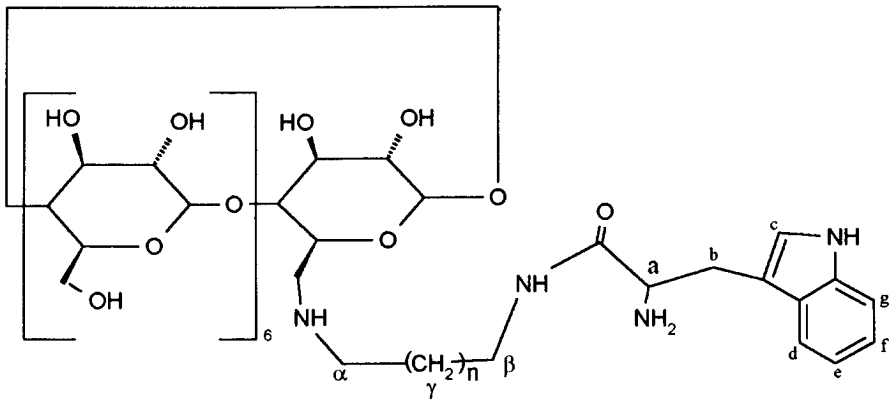
1. Introduction

Cyclodextrins are a class of macrocyclic carbohydrate oligomers that exhibit a number of fascinating physico-chemical and chemical properties [1]. In view of their torus shaped structure with a hydrophobic interior, they are able to act as hosts to a variety of organic molecules, thus forming so-called inclusion compounds [2]. Cyclodextrins and their derivatives are chiral host molecules and they can exhibit enantioselectivity in reaction with a racemic mixture [3–5].

Cyclodextrins have been used as fluorescence enhancing-agents [6] and the competitive binding of a fluorophore is a technique widely used for the determination of the stability constants of cyclodextrin (host–guest) inclusion complexes [7]. The use of a fluorescence probe bound to the cyclodextrin has been developed by Ueno to yield systems in which the fluorescence signal is sensitive to the inclusion process [8]. A large number of cyclodextrin derivatives bearing aromatic molecules have shown intra-molecular inclusion characterised by guest-induced variations in their fluorescence, circular dichroism and absorption spectra [9–17].

Recent reports have described the attachment of amino acids to cyclodextrins via an amide bond, and their intramolecular inclusion using ^{1}H NMR spectroscopy [18–20], fluorescence or circular dichroism [21]. The grafting of amino acids onto the cyclodextrin has been reported to be a good approach to increase the



1 n = 0, L-Trp
2 n = 1, L-Trp
3 n = 0, D-Trp
4 n = 1, D-Trp

Chart 1.

chiral recognition. These kinds of derivatives have been used successfully as chiral receptors through the use of different metals to assist the molecular recognition process [22–24].

In this context, we have synthesised D and L tryptophan modified cyclodextrins, to generate molecules with both a fluorescent probe and a chiral moiety which can effect chiral recognition. We carried out a spectroscopic investigation in order to determine the ability of the derivatives to act as a fluorescent sensor. We used both L and D tryptophan to study the difference generated by chirality.

The derivatives synthesised result from the grafting of L or D-Tryptophan on the 6-deoxy-(1-(2-amino)ethylamino)-β-cyclodextrin [25] (CDen) and the 6-deoxy-(1-(2-amino)propylamino)-β-cyclodextrin [26] (CDpn) (Chart 1).

2. Experimental Section

2.1. MATERIALS

Commercially available reagents were used directly unless otherwise noted: β-CD was dried in vacuo (10 mm Hg) for 24 h at 80 °C using a P_2O_5 trap. TLC was carried out on silica gel plates 60F-254 (Merck). Cyclodextrin derivatives were detected with UV light and the anisaldehyde reagent. Merck lichroprep RP-8 (40-60 mm) was used for reversed phase column chromatography.

Fluorescence spectra were measured on a Jasco FP-777 spectrofluometer using a 10 mm quartz cell. The emission and excitation widths were set at 1.5. Measures of quantum yields were made in water. No smoothing was applied to the spectra.

Electronic and c.d. spectra were recorded on a Beckam DU 650 spectrophotometer and on a JASCO J-600 dichrograph, respectively. Calibration of the c.d. instrument was performed with a 0.06% solution of ammonium camphorsulfonate in water ($\Delta\varepsilon$ = 2.40 at 290.5 nm). Competitive complexation experiments were carried out in H_2O/MeOH 90/10. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Brucker AC-200 MHz spectrometer at 200.13 MHz and at 50.33 MHz respectively, in D_2O without reference compound. ^{1}H NMR spectra for the 1-adamantanol inclusion studies were performed in D_2O/MeOD 90/10 v/v. The addition of the MeOD did not modify the ^{1}H NMR spectra of derivatives.

2.2. SYNTHESIS

The 6-deoxy-6-[1-(2-amino)ethylamino]-β-cyclodextrin [25] and the 6-deoxy-6-[1-(2-amino)propylamino]-β-cyclodextrin were synthesised as previously described [26].

*6-deoxy-6-[1-tryptophanylamidoethylamino]-β-cyclodextrin (**1**)*

The tert-butyloxycarbonyl-L-tryptophan (Trp-BOC) (52 mg) was added to a solution of CDen (200 mg) in DMF in the presence of benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (75 mg) and *N*-l-hydroxybenzotriazole (HOBT) (26 mg) [27]. The reaction mixture was stirred vigorously at room temperature under nitrogen over 2h. The solvent was evaporated to dryness in vacuo, and purified by elution from a column (20 $\times$ 600 mm) of CM-sephadex C-25 (NH_4^+ form) firstly with water (400 ml), then with a 0–0.2 M NH_4HCO_3 linear gradient (total volume 1.1L). The appropriate fractions were concentrated to give CDen-L-Trp-Boc, R_f = 0.46 (PrOH/H_2O/AcOEt/NH_3 5 : 3 : 1 : 1), yield: 65%.

For the deprotecting step, the CDen-L-Trp-Boc was dissolved in neat trifluoroacetic acid. The solution was stirred at room temperature for 1 h and the solvent was evaporated. The product was purified by chromatography as described above. The appropriate fractions were concentrated to give **1**, R_f = 0.39 (PrOH/H_2O/AcOEt/NH_3 5 : 3 : 1 : 1), yield: 85%.

^{1}H NMR (D_2O, 200MHz): δ 1.7–1.8 (m, 2H, Hα), 2.4–2.8 (m, 3H, H-6A, H-6′A, Hβ), 2.93–3.2 (m, 3H, Hb, H′β), 3.3–4.05 (m, 33H, H-4, Ha, H-2, H-5, H-6, H-3), 4.9–5 (m, 6H, H-1), 5.05 (d, 1H, H-1A), 6.85–7 (m, 2H, Hf, He), 7.24 (s, 1H, Hc), 7.31–7.36 (m, 2H, Hg, Hd, $J_{g,f} = J_{d,e}$ = 8 Hz). ^{13}C NMR (D_2O, 50.33 MHz): δ 33 (Cβ), 33.6 (Ca), 41.1 (Cα), 48.3 (Cb), 59.1 (C-6A), 62.9 (C-6), 71.1 (C-5A), 74.3–75.7 (C-3, C-5, C-2), 83.4–84.1 (C-4), 104.2–104.7 (C-1), 112.5, 113.7, 121.6, 124.4, 126.5, 128.5, 138.4 (C of the aromatic ring). FAB/MS 1364 (M + 1) m/z

*6-deoxy-6[L-tryptophanylamidopropylamino]-β-cyclodextrin (**2**)*

The product was obtained following the same procedure previously described for **1** using CDpn, R_f = 0.42 (PrOH/H_2O/AcOEt/NH_3 5 : 3 : 1 : 1), yield: 45%.

^{1}H NMR (D_2O, 200 MHz): δ 1.2–1.5 (m, 2H, Hγ), 2–2.1 (m, 2H, Hα) 2.5–2.9 (m, 4H, H-6A, H-6′A, Hb), 3.01–4.05 (m, 1H, Hβ, Ha, H-2, H-4, H-6, H-5, H-3), 4.9–5 (m, 7H, H-1), 7.16–7.27 (t, 2H, Hf, He), 7.24 (s, 1H, Hc), 7.50 (d, 1H, Hd, $J_{d,e}$ = 7.8 Hz), 7.58 (d, 1H, Hg, $J_{g,f}$ = 7.2 Hz). ^{13}C NMR (D_2O, 50.33 MHz): δ 32.9 (Cβ, Ca), 39.4 (Cγ), 47.2 (Cα), 51.5 (C-6A), 58.9 (Cb), 62.6–63 (C-6), 71.4 (C-5A), 74.8–75.8 (C-3, C-5, C-2), 83.6–84 (C-4), 86.9 (C-4A), 104.7 (C-1), 112.6, 114.2, 120.6, 122.1, 124.6, 126.5, 129.8, 139 (C of the aromatic ring). FAB/MS: 1378 (M + 1) m/z.

*6-deoxy-6[D-tryptophanylamidoethylamino]-β-cyclodextrin (**3**)*

The product was obtained following the same procedure previously described for **1**. R_f = 0.41 (PrOH/H_2O/AcOEt/NH_3 5 : 3 : 1 : 1), yield: 56%.

^{1}H NMR (D_2O, 200 MHz): δ 1.98 (m, 2H, Hα), 2.4–2.55 (m, 2H, H-6A, H-6′A) 2.8–3.1 (m, 4H, Hβ, Hb), 3.25–4.1 (m, 9H, H-4, Ha, H-2, H-5, H-6, H-3), 4.9–5.07 (m, 7H, H-1), 6.99–7.01 (m, 2H, Hf, Hg), 7.24 (s, 1H, Hc), 7.36 (d, 1H, Hd, $J_{d,e}$ = 7.6 Hz), 7.43 (d, 1H, Hg, $J_{g,f}$ = 6.8 Hz). ^{13}C NMR (D_2O, 50.33 MHz): δ 32.9 (Cβ), 33.4 (Cb), 41.6 (Cα), 58.3 (Ca), 60.9 (C-6A), 62.9 (C-6), 72.3 (C-5A), 74.6–75.9 (C-3,C-5,C-2), 83.5 (C-4), 104.5 (7C, C-1), 106, 112.1, 114.2, 121.1, 121.8, 124.4, 129.8, 138.8 (C of the aromatic ring). FAB/MS: 1364 (M + 1) m/z.

*6-deoxy-6[D-tryptophanylamidopropylamino]-β-cyclodextrin (**4**)*

The product was obtained following the same procedure previously described for **1**. R_f = 0.39 (PrOH/H_2O/AcOEt/NH_3 5 : 3 : 1 : 1), yield: 52%.

^{1}H NMR (D_2O, 200 MHz): δ 1.33 (m, 2H, Hγ), 2.15–2.7 (m, 2H, Hα), 2.6–3.15 (m, 2H, H-6A, H-6′A, Hb, Hβ), 3.3–4.12 (m, Ha, H-2, H-4, H-6, H-5, H-3), 4.9–5.04 (m, 7H, H-1), 7.08 (m, 2H, Hf, Hg), 7.23 (s, 1H, Hc), 7.42 (d, 1H, Hd, $J_{d,e}$ = 7.6 Hz), 7.52 (d, 1H, Hg, $J_{g,f}$ = 8 Hz). ^{13}C NMR (D_2O, 50.33 MHz): δ 33.7 (Cb, Cβ), 39.4 (Cγ), 46.7 (α), 50.7 (C-6A), 58.9 (Ca), 63 (C-6), 70.9 (C-5A), 74.8-75.9 (C-3, C-5, C-2), 83.6 (C-4), 86.5 (C-4A), 104.7 (7C, C-1), 112.6, 114.4, 120.5, 122.2, 124.7, 126.4, 129.8, 138.9 (C of aromatic ring), 178.9 (CO). FAB/MS spectrum: 1378 (M + 1) m/z.

3. Results and Discussion

3.1. SYNTHESIS

The CDen and the CDpn were obtained in two steps from the parent cyclodextrin as described elsewhere [25,26]. For derivatives **1**–**4** (Chart 1), the tryptophan (D or L), protected by the ter-butyloxycarbonyl group, was grafted to cyclodextrin

derivatives using benzotriazolyl-N-oxytris(dimethylamino) phosphonium hexafluorophosphate (BOP) with N-l-hydroxybenzotriazole (HOBT) in DMF at room temperature [27]. The products were deprotected in neat trifluoroacetic acid and purified by chromatography. The purity was checked by ^{1}H NMR.

3.2. NMR SPECTROSCOPY

NMR data confirmed the identities of our compounds. The ^{1}H NMR spectra were assigned on the basis of the COSY spectra.

As for derivative **1**, the aromatic region can easily be assigned as being between 7 and 7.4 ppm. Substitution in β-CD causes the shift of the signals of the protons of the substituted ring. In addition to the peaks typically observed on the parent CD spectrum, the peaks of the amine and of the tryptophan can be seen. On the spectrum the inequivalence of the H-1 region is evident, as a consequence of the functionalization. As for the region upfield, the spectra can be attributed on the basis of the COSY spectra (Figure 1). At 1.73 ppm the broad peaks due to the Hα can be seen. The two Hα are diastereotopic. On the COSY spectra, two cross peaks with Hβ and H'β signals at 2.7 and 3.2 ppm are present, showing that the two Hβ are diastereotopic, too. These data suggest rigidity of the ethylenic chain that can be related to the presence of the tryptophan. In the CDen previously investigated [25,28] the protons were not shown to be diastereotopic. At 2.5 and 2.8 ppm the peaks of the two diastereotopic H-6A are present. They have shifted upfield as a consequence of the functionalization. Both the H-6A show a cross peak with the H-5A, which resonates at about 3.5 ppm, in the H-2,4 region. The ABX pattern of the tryptophan is evident on the COSY spectra: the Ha peak is at 3.6 ppm and the cross peaks with both the Hb are shown on the COSY spectrum. For the other derivatives, the spectra are on the whole similar to derivative **1**, but some differences can be underlined.

The signals of the aromatic protons showed a large modification in comparison with the tryptophan alone. While in derivative **1** the aromatic protons Hd and Hg showed a single doublet, for **2**, **3** and **4**, and for tryptophan alone, these protons showed two doublets (Figure 2). This effect could suggest a peculiar orientation of the indole in derivative **1**. The two Hα protons of **3** are not diastereotopic and show a slightly downfield shift in comparison with derivative **1**, as shown in the COSY in Figure 3. The spin-coupled Hβ with Hα protons are less diastereotopic than the same protons in derivative **1**, thus suggesting a less rigid ethylenic chain. In the spectra of derivatives **2** and **4**, shown in Figures 4 and 5 respectively, the Hα protons absorb at 2.2 ppm, and in derivative **2** they are more diastereotopic than in derivative **4**, as was the case for derivatives **1** and **3**. The Hβ protons are not diastereotopic in these derivatives, suggesting less rigidity in the propylenediamine derivatives. The longer chain modifies the disposition of the functionalizing moiety. Another difference is the chemical shift of the H-5A. While in the case of derivatives **1** and **3** the chemical shift is at about 3.5 ppm, in the case of derivatives **2** and **4** it is shown

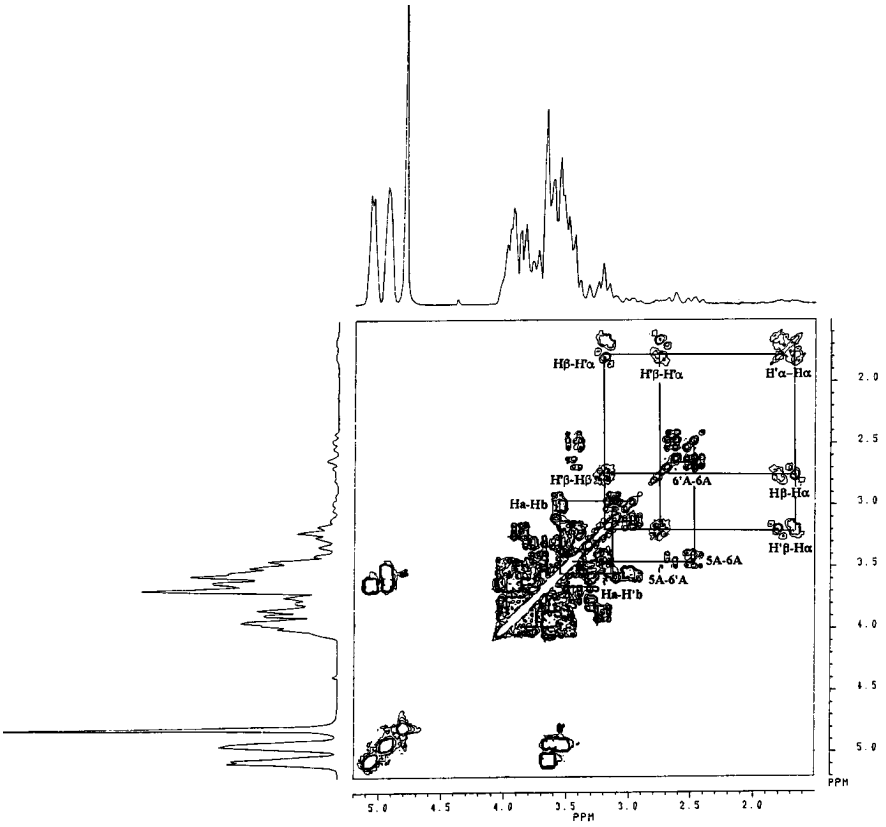


Figure 1. COSY spectrum of **1** (5.2–1.5 ppm region, 200 MHz, D_2O).

at about 4.0 ppm as typically observed in the CDen or other monofunctionalized derivatives. The resonance of the H-3 and H-5 protons of the cyclodextrin are known to shift upfield or downfield in response to the depth of inclusion, this shift being due to the ring current effect of aromatic ring [18]. For our compounds, the resonance of the H-5A proton is largely shifted downfield for derivatives **1** and **3**, while for derivatives **2** and **4**, no shift occurs in comparison with the diamine-derivatives (CDen, CDpn) [25,26]. The H-3A remains at about 3.9 ppm, as in the CDen or CDpn derivatives. These data, together with the upfield shift of the Hα and the diastereotopicity of these protons, particularly in **1**, suggest the inclusion of the indole in the cavity. The indole ring should be near to the Hα and thus its current shifts the signal of the Hα and of the H-5A upfield. These are approximately on a plane perpendicular to the plane containing the indole ring. The H-3A is not influenced by the ring current effect because, as the CPK models suggest, the ethylene chain does not allow a deeper inclusion of the ring. In the case of **2** and **4**, the longer chain seems to reduce the rigidity of the system. No direct information on the inclusion of the indole are available on the COSY spectra. The differences in the

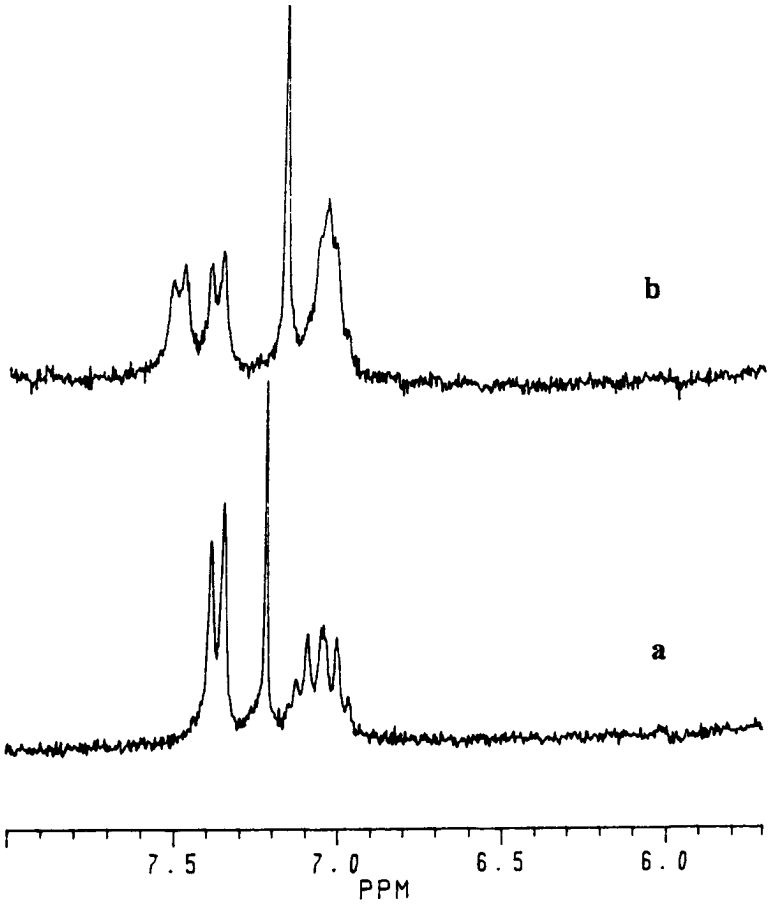


Figure 2. Partial ^{1}H NMR spectra (200 MHz) of **1** (5.10^{-3} M) in D_2O/MeOD alone (a) and in the presence of 1-adamantanol (5×10^{-3} M) (b).

aromatic region strongly suggest that in derivative **1** the indole can interact strongly with the interior of the cavity. On the other hand, a recent investigation has shown that though aromatic protons of the L-tryptophan derivative of the cyclodextrin exhibit a large chemical shift in comparison with the tryptophan alone, it does not show self-inclusion, but rather a specific orientation outside the cavity [20]. Further proof of the self-inclusion has yet to be provided in competition experiments.

In order to ascertain intramolecular inclusion, the 1-adamantanol was used as a competitive guest [4]. The ^{1}H NMR spectra were carried out in the presence of 1-adamantanol in water methanol 90/10. The chemical shifts are provided in Table I, for a 1 : 2 host–guest ratio.

All derivative spectra are more or less modified upon addition of adamantanol (see Table I). It is known that the aromatic signals (Hg, Hd and Hc) of the free tryptophan are shifted upfield upon inclusion in the cavity of the α-cyclodextrin

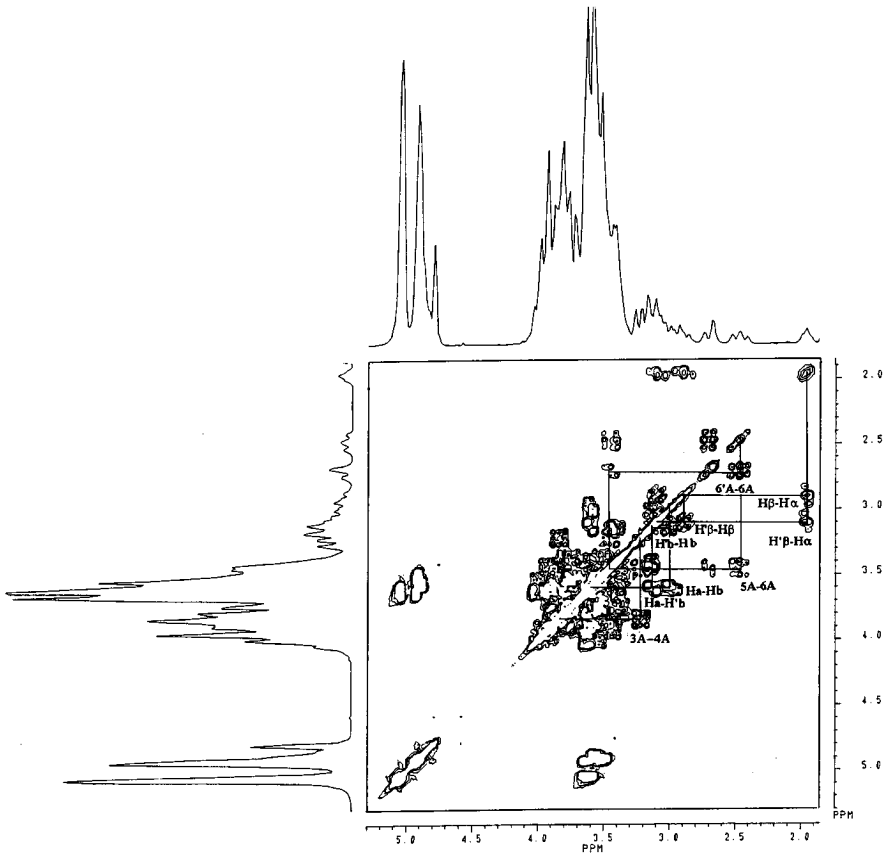


Figure 3. COSY spectrum of **3** (5.2–1.8 ppm region, 200 MHz, D_2O).

Table I. Tabulation of chemical shifts (200 MHz, Hz). [CD] = 5×10^{-3} M, [1-adamantanol] = 5×10^{-3} M in D_2O/MeOD 90 : 10.

	$\Delta\delta$ (**1**)	$\Delta\delta$ (**2**)	$\Delta\delta$ (**3**)	$\Delta\delta$ (**4**)	$\Delta\Delta\delta$ (**1–3**)	$\Delta\Delta\delta$ (**2–4**)
Hc	16.3	−0.3	1.0	6.9	15.3	−7.2
Hg	−34.0	0.2	−30.6	0.6	−3.4	−0.3
Hd	−11.6	10.0	−13.5	18.4	1.9	−8.3
He, Hf	−11.8	25.5	1.7	28.8	−13.5	−3.3

[29]. For derivative **1** (Figure 2) all the protons are modified, the chemical shift of the Hc proton undergoes a small variation; the signals of Hg, Hf, He and Hd are shifted downfield upon guest addition; while the signal of Hc is shifted upfield. For derivative **3**, the Hg and Hd protons show the same shift as in derivative **1**, while Hc, Hf and He show a very low upfield shift.

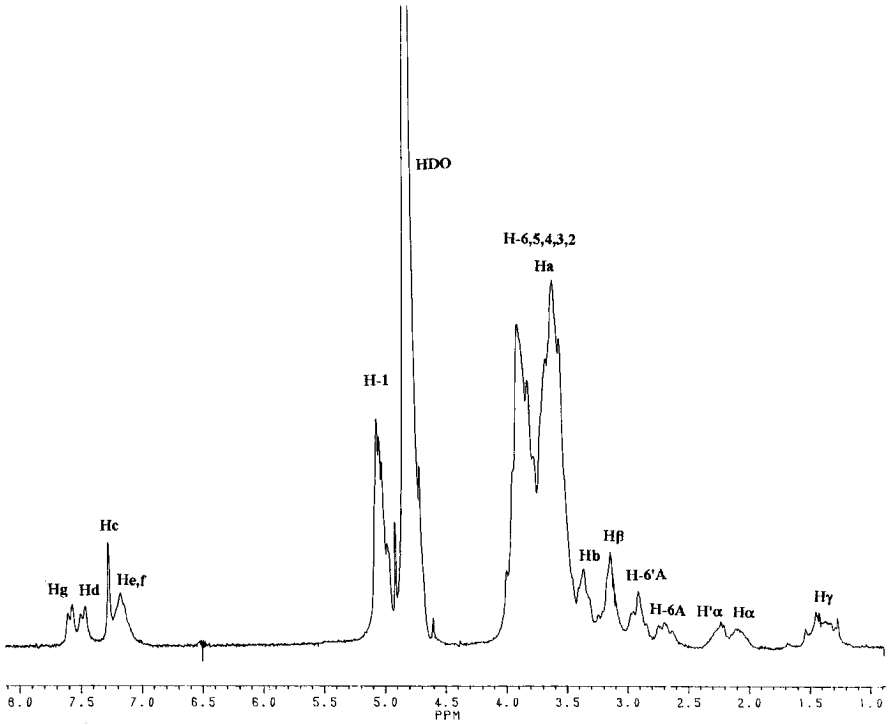


Figure 4. ^{1}H NMR spectrum of **2** (200 MHz, D_2O).

For derivative **4**, the aromatic signals are shifted upfield corresponding to the indole moiety being driven out of the cyclodextrin cavity. The Hf and He protons show the greatest changes. Derivative **2** shows the smallest modification in the chemical shift of Hc and Hg, probably because there are fewer interactions between the cavity and the indole, except for the signal of the Hf and He protons. The large chemical shift variation of these protons suggests that the indole is localised near the cavity allowing interactions between the Hf and He and the protons of the cyclodextrin. Derivatives **2** and **4** show the same behaviour, suggesting a similar position of the indole ring. The difference of the chemical shifts ($\Delta\Delta\delta$) in the L and D derivatives are greater for the CDen derivatives than for the CDpn. These results suggest that for all these derivatives, the indole could be included. Derivative **1** seems to have a larger affinity to form an intramolecular complex. The ethylenic chain seems to promote the self-inclusion.

3.3. FLUORESCENCE

Fluorescence quantum yields of the derivatives were measured, using an acid aqueous solution of quinine sulphate as standard [30], and were compared to tryptophan values measured in the same conditions. Quantum yields and maximum

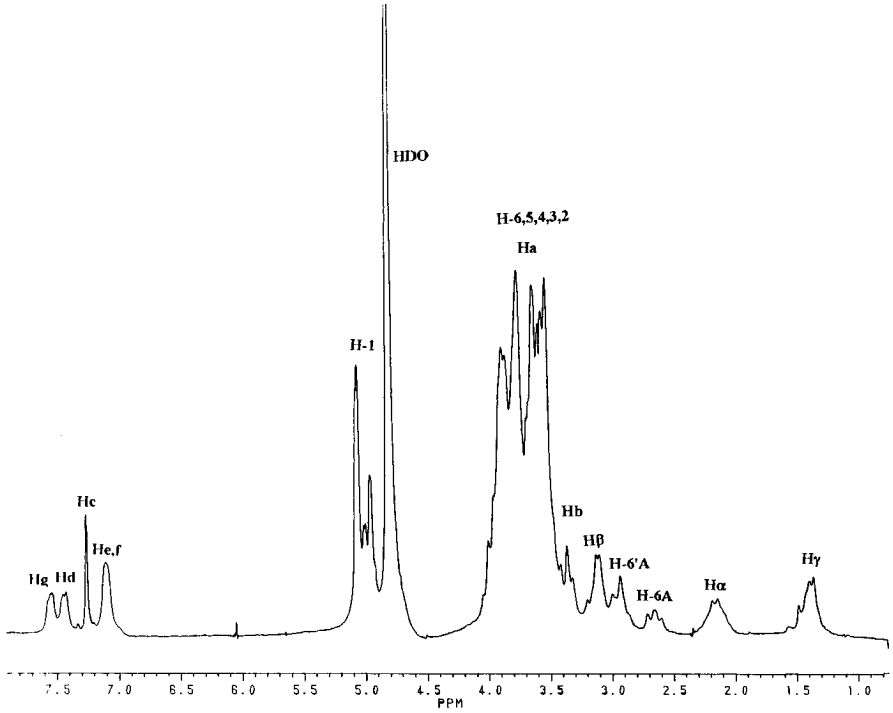


Figure 5. ^{1}H NMR spectrum of **4** (200 MHz, D_2O).

Table II. Quantum yields and maximum wavelengths of derivatives.

	Φ_F	λ_{max} (nm)
L-Tryptophan	0.18	359
1	0.48	343
2	0.54	350
3	0.56	347
4	0.49	348

wavelength emission are listed in Table II. Fluorescence, which is sensitive to the immediate environment of the tryptophan, gives information on the position of the indole in the cavity of the cyclodextrin. The fluorescence spectra of the indole show a pronounced red shift in polar solvents compared to its fluorescence in apolar solvents [31]. The cyclodextrin cavity behaves like an organic solvent, it provides an apolar environment for the included chromophores. Indole fluorescence is enhanced upon inclusion in the cyclodextrin and its maximum wavelength emission is blue-shifted [32,33].

Maximum wavelength emissions of derivatives **1**–**4** show a blue shift compared to the maximum wavelength emission of the amino acid alone. Furthermore, all the

compounds show quantum yields higher than for tryptophan. These observations suggest the strong modification of the polarity experienced by the indole in the cyclodextrin cavity. Fluorescence is enhanced by the relatively hydrophobic cavity giving higher quantum yields and a blue shift of the maximum wavelength emission. The values do not vary according to the concentration of the derivatives, which leads us to propose that self inclusion occurs in all cases. The maximum wavelength emission of derivative **1** shows the largest shift, suggesting the deeper inclusion of the indole. Derivative **2** shows the shorter blue shift of the maximum wavelength emission, suggesting a less deep inclusion of the indole in the cavity.

This assumption is supported by competition experiments. Figure 6 shows the fluorescence spectra of derivatives **1**–**4**, alone and in the presence of 1-adamantanol, respectively. Fluorescence intensity changes markedly upon guest addition for derivatives **1**, **3** and **4** indicating the strong modification of the environment of the indole upon its being driven out of the cavity in a polar solvent. Tryptophan fluorescence is enhanced within the cyclodextrin cavity, the guest takes the place of the indole ring inducing fluorescence to decrease. This is further confirmed by the red-shift of the maximum wavelength emission of these derivatives upon guest inclusion, suggesting that the indole is driven out of the cavity. Derivative **2** shows fluorescence intensity to diminish slightly, suggesting the less deep inclusion of the indole in the cavity in comparison with the other derivatives, as seen before. The largest decrease in fluorescence intensity is observed for derivative **1**, and the difference in intensity variations between L and D derivatives is larger for the CDen-modified cyclodextrins. The fluorescence intensity of derivative **1** diminishes to 32% and to 12% for derivative **3**.

3.4. CIRCULAR DICHROISM

The interactions between guest and derivatives have also been studied using circular dichroism. Figure 7 shows the c.d. spectra of derivatives **1**–**4**, alone and in the presence of 1-adamantanol. The inclusion of a chiral molecule in the cyclodextrin cavity modifies the c.d. spectrum of the guest. This is dependent on the position of the guest in the cavity [34]. The same considerations can be made in the case of an intramolecular inclusion of the chiral moiety. The c.d. spectra of derivatives **1** and **3** are similar with a positive band due to the tryptophan residue at 222 nm and a wide positive band at 285 nm. If the L-Trp alone is taken qualitatively as the reference compound, the c.d. spectrum of derivative **1** shows a less positive effect in comparison. The spectrum of derivative **3** has a positive band at 222 nm, in contrast with the D-tryptophan alone. The Cotton effect for derivative **3** would appear to be more significant than that seen for derivative **1**. This suggests that the indole is differently situated in the cavity. The modification of $\Delta\epsilon$ for derivatives **1** and **3** may suggest axial inclusion in the case of derivative **3** [35] and a lid-type inclusion of the indole ring in the case of derivative **1** [36]. When derivative **3** is in the presence of the 1-adamantanol, the intensity of the two bands diminishes and the spectrum

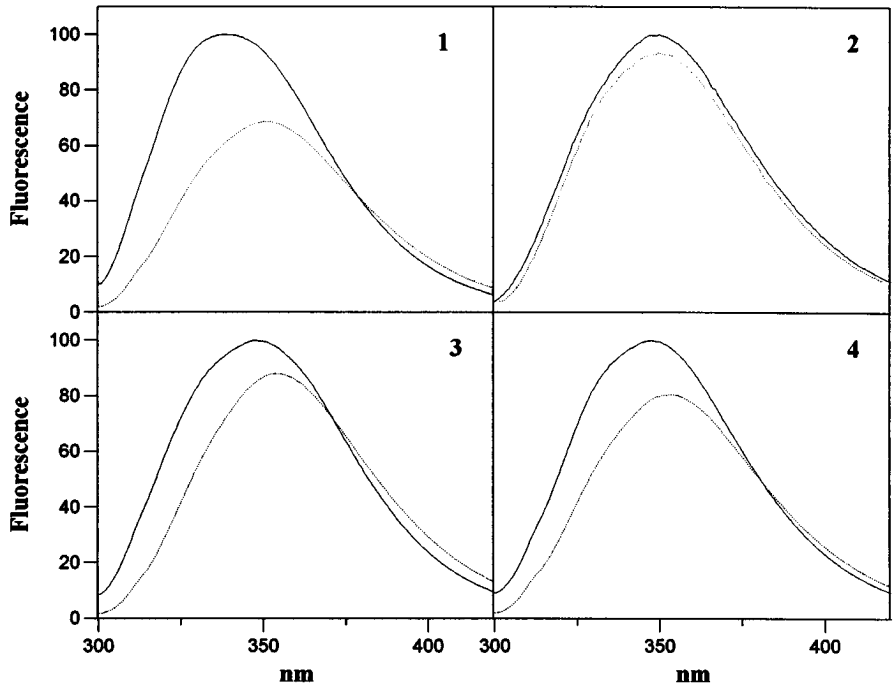


Figure 6. Fluorescence spectra of **1**–**4** alone (——) and in the presence of 1-adamantanol (- - - -) in a solution water-methanol 90 : 10. [CD] = 1×10^{-5} M.

obtained is the enantiomeric image of derivative **3** alone. In the case of derivative **1**, a slight increase in intensity of the band at 222 nm was observed in the presence of the 1-adamantanol. It may be presumed that the indole is disincluded in both derivatives, but in the case of derivative **1**, it takes a new conformation inducing a Cotton effect with the same sign, as described for other derivatives [37]. The c.d. spectra for derivatives **2** and **4** are similar to those of derivatives **1** and **3**. Both derivatives show a positive band at 222 nm and after the addition of 1-adamantanol the spectra become quasi enantiomeric. This result is consistent with the indole being displaced from the cavity. The c.d. spectra suggest that the longer propylene chain moves the indole residue away from the cavity, which is not evident in the ethylenediamine derivatives.

4. Conclusion

These results suggest that there is a direct relationship between the features of these systems and their structure. The disposition of the amino acid in the cavity depends on the chirality of the amino acid, and on the length of the chain. If we compared derivatives L and D, large differences in sensitivity as regards the interaction with the guest 1-adamantanol were observed, for both the CDen or CDpn derivatives.

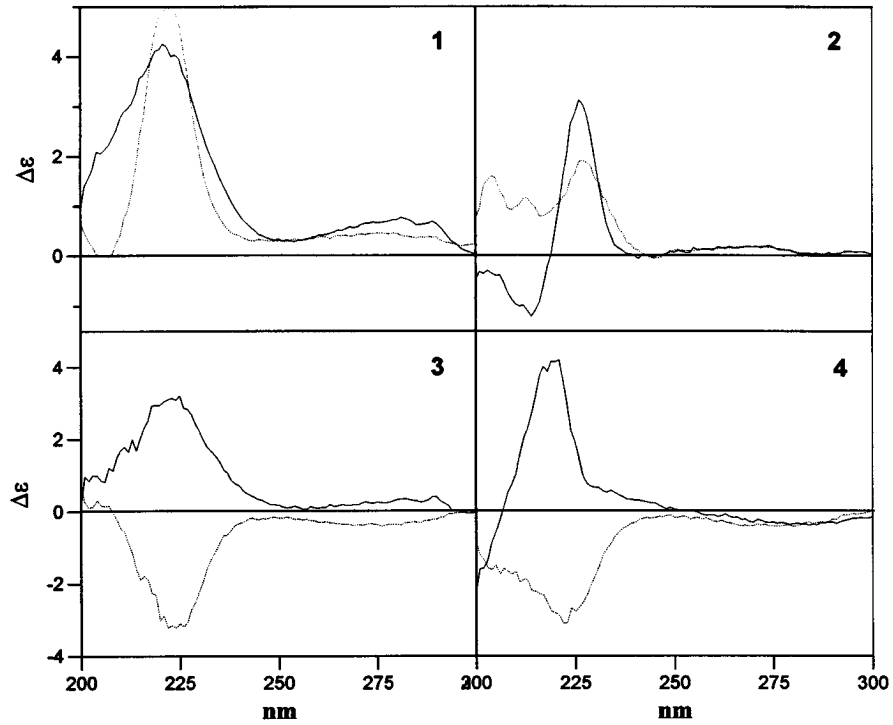


Figure 7. Circular dichroism spectra of **1**–**4** alone (—) and in the presence of 1-adamantanol (- - - -) in a solution of water/methanol 90/10.

Derivative **1** seems to have a greater tendency to form an intramolecular complex. This derivative shows the higher modification of fluorescence, c.d. and NMR spectra. On the basis of the fluorescence and c.d. spectra, derivative **2** might not be included in the cavity of the cyclodextrin, but in the upper part of the cavity instead. The NMR spectra with the competitive guest confirm this result, since the NMR spectra for this system undergo the slightest changes, involving only the He and Hf protons.

In the previously described example of chiral intramolecular recognition in cyclodextrin derivatives, short spacers between the host and the intramolecular guest seemed to force partial inclusion of the latter [9]. In our cases, the presence of the chain gives rise to a system with greater conformational flexibility, allowing the deeper inclusion of the indole within the cavity. The additional presence of the chain between the cyclodextrin and the indole also provides molecules more sensitive to detection by fluorescence than the derivatives obtained with the direct grafting of the tryptophan on to the cyclodextrin [21]. The studies show that tryptophan-modified-cyclodextrins are good sensory systems that exhibit spectral variations upon guest inclusion detected both by circular dichroism and by fluorescence. The derivatives may be used for detecting organic compounds through fluorescence variations, even

if the compounds are optically inert. Furthermore, in comparison with cyclodextrins functionalized with an achiral fluorophore, the chiral centre of the amino acid may improve chiral recognition ability and can allow the enantioselective detection of chiral compounds.

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