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Specific Interaction and Stabilization between Host and Guest : Complexation of Ellipticine in a Nucleobase Functionalized Cyclodextrin.

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Abstract : A nucleobase derivative of β-cyclodextrin was used to form a highly soluble inclusion complex of ellipticine. The reality of inclusion and the formation of a π-π interaction between host and guest were established by NMR.

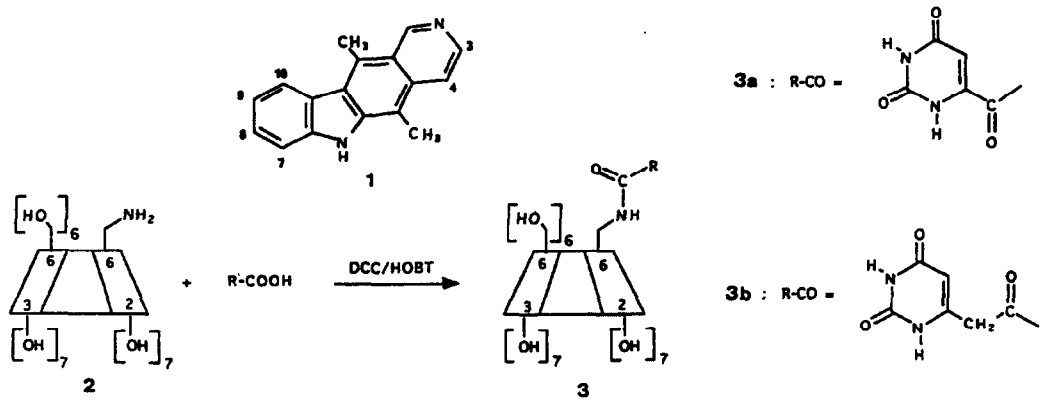
Although promising as a broad scope anticancer agent [1], the interest for the ellipticine series has dropped in relation with the insolubility of ellipticine and of active derivatives in aqueous solutions. Three ways have been attempted to increase its solubility to therapeutically relevant values:

- Quaternarization of the pyridyl nitrogen has led to 9-hydroxy-2-methyl ellipticinium acetate of therapeutic interest [2].
- Glycosyl derivatives of ellipticine have been prepared [3].
- Complexation in γ-cyclodextrin has been reported but the solubility of the inclusion complex remains very low and this compound has been investigated by fluorescence techniques in hydro-alcoholic solutions [4].

Chemical modification of cyclodextrins can lead to an improvement of their inclusion capacity or specificity[5]. For example, partial methylation can increase the solubility of inclusion complexes and capping the primary alcoholic side [6] or both sides[7] was shown, in some cases, to improve the inclusion properties. Hydrophobic interactions between the guest and the relatively non polar cavity are in most cases advocated to explain the formation of inclusion complexes with cyclodextrins. We wish to present here the first case of additional stabilization of a host-guest inclusion complexes by formation of a π-π donor-acceptor pair.

Nucleobase functionalized β-cyclodextrin has already been described [8] but has not been used as inclusion media for intercalating agents. Compounds **3a** and **3b** (scheme 1) have been prepared by acylation of 6-amino-6-deoxy-β-cyclodextrin **2** using the dicyclohexyl-carbodiimide/hydroxybenzotriazole (DCC/HOBT) procedure in DMF at room temperature as described elsewhere [9]. The complexation studies have been performed with derivatives **3** [10]. Unmodified β-cyclodextrin and heptakis(2,6-di-*O*-methyl) cyclomaltoheptaose were also used but did not lead to any improvement of the solubility of ellipticine **1**.

The solubility of ellipticine in aqueous solutions of compounds **3a,b** was deduced from both optical measurements and NMR data. In the presence of **3a**, aqueous solution containing up to 5 $mg.mL^{-1}$ ellipticine could be obtained. Conversely, derivative **3b** displays by far poorer solubilization properties which remain in the range available by use of γ-cyclodextrin (ca. 0.1 $mg.mL^{-1}$). All NMR experiment were carried out on derivative **3a**.



Scheme 1.

NMR experiments.

In a first step, NMR was used to evidence the formation of an inclusion complex between the ellipticine molecule and the cyclodextrin. The effects of inclusion of aromatic compounds in cyclodextrins are well documented [11]. Upfield shifts are expected for protons H3 and H5 of the host as induced by ring current effects of the guest. In the present case, reduction of the symmetry of the host leads to complex spectra with virtually different spectral properties for all seven units. The addition of ellipticine leads to clear variations of the NMR spectrum but they cannot be attributed to specific protons of the host. For this purpose, 2D NMR experiments were carried out to identify proton classes. These techniques rest upon the stepwise assignment of protons of the glucose units starting for the isolated anomeric protons[12].

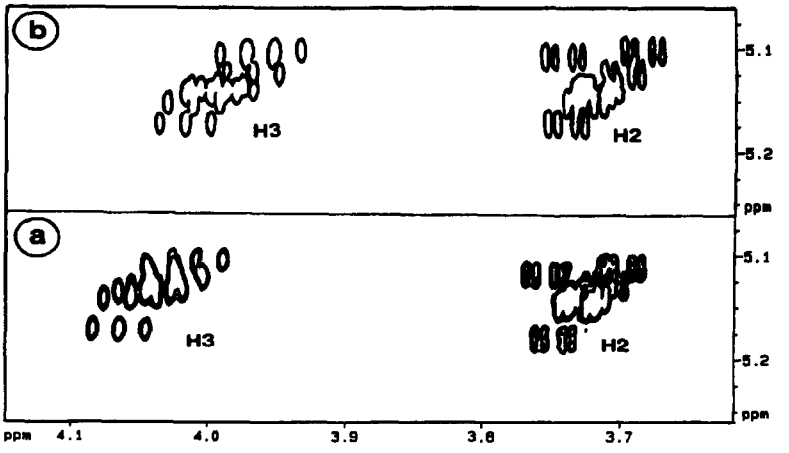


Figure 1

Figure 1 (500 MHz, 298K, D_2O) shows the partial contour plots from one-step relay experiments (transfering information from H1 to H3) were protons H3 (appearing individually as triplets) are clearly identified.

Comparison of the chemical shifts of these protons in **3a** alone (a) and in the presence of 3mM ellipticine (b) shows significant upfield shifts (although H2 protons remain virtually unaffected) indicating inclusion of the aromatic ring of ellipticine in the cavity.

In a second step, NMR was used to investigate the behaviour of the aromatic moieties. Important conclusions can be derived from the simple observation of the 1D NMR spectra. Figure 2 displays the partial spectra (aromatic domain) of **3a** in the absence (a) and in the presence of 2mM (b) and 3 mM (c) ellipticine.

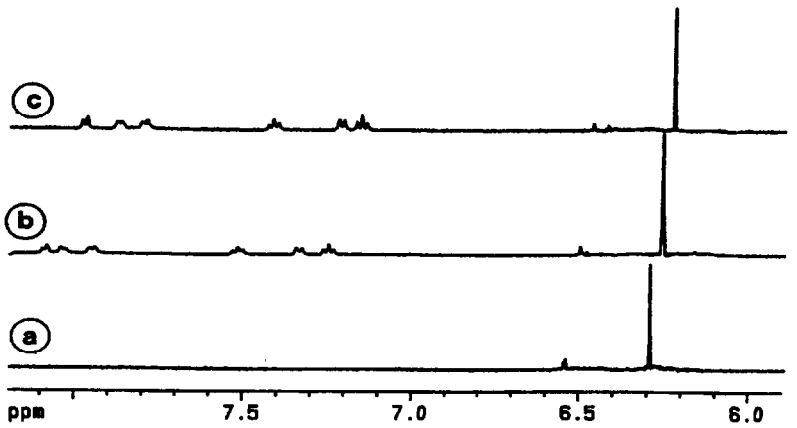


Figure 2

Two observations can be derived.

- First, large concentration dependent variations of the chemical shifts of aromatic protons of ellipticine are observed. These effects are mainly due to the self-association of the drug which induces upfield shifts upon increasing concentration.
- Second, a clear upfield shift of the aromatic proton of the nucleobase (at 6.3 ppm in free **3a**) is evidenced upon addition of ellipticine. This shift is up to ca. 0.08 ppm upfield for 3 mM ellipticine in the presence of 10 mM **3a**. This observation is in agreement with the formation of a charge-transfer complex. The simultaneous presence of auto-association and inclusion processes precludes the safe determination of the association constant using simple interaction models.

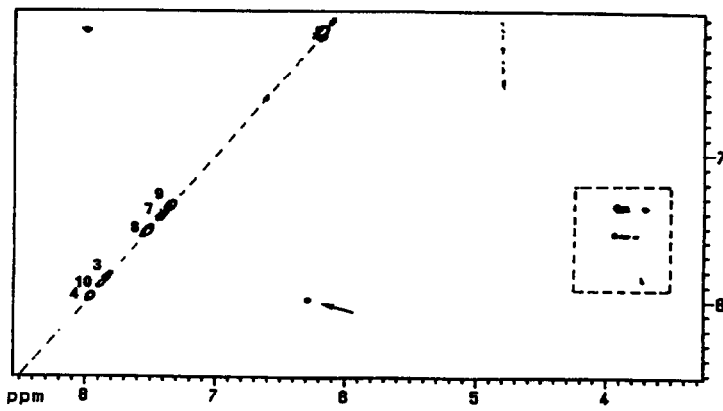


Figure 3

Finally, the reality of these interactions was evidenced by a bidimensional ROESY experiment [13] dedicated to show spatial proximities between protons of the host and guest. A partial contour is presented in Figure 3 (500 MHz, 298K, 3 mM ellipticine, 10 mM **3a**). In addition to cross-peaks between proton 7-9 of ellipticine and signals from the cyclodextrin (dotted box), a sharp peak (indicated by an arrow) is observed between the aromatic proton of the nucleobase and proton H4 of ellipticine as expected from the formation of the charge transfer complex. The geometry of the complex involves partial penetration of the indole moeity in cyclodextrin and stacking of the remaining of the molecule with the nucleobase.

The data presented clearly indicate that combination of non-specific and guest-specific interactions can lead to a considerable improvement of the inclusion and solubilization properties of cyclodextrins. The efficiency of such processes is, however, highly demanding in terms of optimized adaptation of the two molecules. This is clearly indicated by the fact that derivative **3b** does not improve the inclusion and solubilization properties of ellipticine as compared to unmodified cyclodextrins. Obviously, the presence of an additional methylene group between the cyclodextrin and the nucleobase precludes the formation of a stable charge-transfer complex. In this case, the cumulative effects of inclusion and stacking with the nucleobase do not hold. This observation indicates that molecular modelling can play a key role in the design of optimized carriers for a specific series of potential guest molecules. In this respect, the integrated use of molecular modelling, chemical modification and NMR investigations is expected to open the field of new specific drug-carriers dedicated to vectorization of drugs of high potential therapeutic value.

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10. Preparation of the complex : A suspension of ellipticine in methanol was added to a solution of the cyclodextrin derivative in 5 mL water. The mixture was vigorously shaken for 30 minutes and concentrated *in vacuo*. The remaining solid was taken up in 2 mL water, filtered and freeze-dried. For NMR experiments it was redissolved in deuterium oxide.

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