Spectroscopic Characterization of the System β-Cyclodextrin + Propafenone Hydrochloride + Water

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Propafenone hydrochloride (PRF) and the complex that forms with β-cyclodextrin (β-CD) in water have been investigated by different spectroscopies: 1D and 2D ^{1}H NMR, steady-state fluorescence, and absorption spectroscopy. Fluorescence measurements show the formation of a complex with a quantum yield lower than that of the pure PRF, although still fluorescent. The resultant quenching is mainly static in nature, as deduced from the temperature dependence of the emission and from the absorption spectra. The decrease in fluorescence has been used to assess the formation constants by nonlinear regression analysis and, from their dependence on the temperature, to obtain the ΔH and ΔS of the binding. All the resonances of the proton NMR spectrum of PRF in D_2O have been assigned. The plot of the induced chemical shifts for certain protons of both molecules versus the mole ratio indicate a 1:1 stoichiometry, and their analysis yields a formation constant that is in perfect agreement with that obtained using molecular luminescence. ROESY experiments, assisted by molecular modeling strategies, reveal the most likely structure of the complex in solution, in which both phenyl groups are buried into the β-CD, with the aliphatic part of PRF protruding the cavity. A slight inclusion of the terminal methyl group is also detected.

Introduction

The microencapsulation by organic hosts of small molecules to form supramolecular assemblies has been revealed as an effective medium to change some of the properties of the substrate (solubility, bio-availability, resistance against radiation, or the attack of reactants, and so on), without modifying its chemical identity. Noncovalent forces between the interacting species, such as van der Waals, hydrogen bonding and dipole−dipole, are responsible for the complexation, constituting the basis of the discipline known as supramolecular chemistry.[1] Whereas a considerable number of molecules are able of acting as the carrier, those that can be used for human consumption are scarce. One of these is β-cyclodextrin (β-CD), which belongs to a family of cyclic oligosaccharides built out of six, seven, or eight α-D-glucopyranose residues, linked by glycoside bonds α-1,4 (α, **β**, and γ-CD respectively). This class of carbohydrates exhibits a doughnut shape, in which the cavity has a hydrophobic character compared to water, whereas the rims, bearing the OH groups, are hydrophilic (Figure 1). Thus, a CD constitutes a singular microenvironment where molecules with suitable size and hydrophobic character can be housed, with all the potential applications that this fact implies.[2]

The mechanism of the complexation comprises the transfer of a molecule, or part of it, to the cavity of a CD. In this process, several effects dependent on the implicated species must be considered, and it is not always easy to identify the chief driving force of the binding. In aqueous media, the release of the water molecules contained in the CD cavity and the dehydration of the ligand, with the subsequent changes in entropy and enthalpy, seem to play an important role.[3]

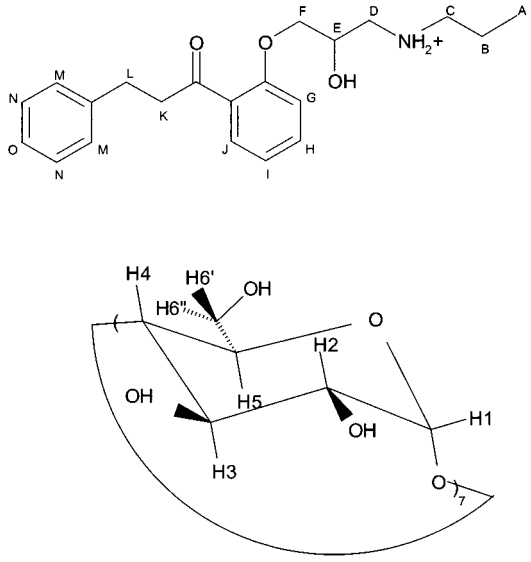

Figure 1. Chemical structures of propafenone hydrochloride (PRF) and β-cyclodextrin (β-CD).

In this investigation, we have selected β-CD as the host and propafenone hydrochloride (PRF) as the guest. Propafenone hydrochloride ($C_{21}H_{28}ClNO_3$) is an anti-arrhythmic agent used

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for the management of severe ventricular and supraventricular arrhythmias. It is normally administrated by mouth, although it may also be given by slow intravenous injection or by infusion. The preparation, pharmacology, uses and administration, and adverse effects are well described in the literature.[4] From the chemical point of view, PRF can be considered a derivative of 3-phenyl-1-propanone (Figure 1). It is soluble in basic lower aliphatic alcohols, CCl_4, and hot water, slightly soluble in cold water, poorly soluble at a basic pH, and insoluble in ether. Since one of its adverse effects is the gastrointestinal intolerance, the microencapsulating properties of cyclodextrins (CDs) could be taken advantage of as a potential way to reduce or avoid these complications and, at the same time, to enhance its solubility. CDs have been used successfully to these purposes with a variety of drugs.[5]

The only precedent of CD−PRF systems is an investigation about the possibility of the enantiomeric resolution of racemic PRF with β-CD, by using capillary electrophoresis (CE).[6] Due to the chiral character of CDs, they are most popular as chiral stationary phases in HPLC and CE.[7] Apart from this one, we have not found physicochemical studies of the interactions of CDs with PRF.

In this paper, the association process in which the complex PRF:β-CD is formed and its structure in solution have been investigated. From the structural viewpoint, PRF is a rather complicated molecule, with two well distinct moieties: an aromatic one, having two phenyl rings in a flexible conformation, and an aliphatic, charged chain. In principle, both of them could be included into the CD. In this sense, 1D and 2D ^{1}H NMR spectroscopies, together with the aid of molecular modeling, are essential tools in order to elucidate the complex structure and to give an idea about the possible inclusion path. Fluorescence and absorption spectroscopies inform about the processes of absorption and emission of energy and allow the calculation of the binding constant and, from their temperature dependence, the thermodynamic parameters of the reaction, i.e., enthalpy and entropy. The pieces of information that each technique contributes separately are brought together in this investigation to give a deep insight of both the complexation process and the structure of the complex in solution.

Experimental Section

Materials. The β-CD was purchased from Roquette, with a purity of 99% and a water content of 13.89%, as determined by thermal analysis. Propafenone hydrochloride, 2′-(2-hydroxy-3-propylaminopropoxi)-3-phenylpropiophenone hydrochloride, was a gift from Rhône Poulenc, with purity higher than 99%. The aqueous solutions for fluorescence and absorption studies were prepared with deionized water from a Millipore Q-System (conductivity lower than 16 μS cm^{-1}).

The samples for ^{1}H NMR were prepared using D_2O as the solvent (Merck and S.d.S., with a deuterium degree higher than 99.9%). In these experiments, the concentration of the drug was kept constant at 0.004 M and that of β-CD varied by adding stock solution of PRF to vials with different amounts of β-CD.

^{1}H NMR Measurements. 1D ^{1}H NMR and COSY studies were carried out with a Varian VXR 300S spectrometer, operating at 300 MHz, at 22 ± 0.1 °C. The digital resolution was 0.2 Hz, and 64 spectra were averaged in each run. The center of the solvent signal (HDO, at 4.792 ppm) was used as the reference. The ROESY spectrum was undertaken in a Bruker 400 MHz spectrometer using a mixing time of 600 ms, on a sample having concentrations of PRF and β-CD of 4 and 6 mM, respectively.

Fluorescence and Absorption Measurements. Steady-state fluorescence measurements were performed with a Perkin-Elmer LS-50B spectrofluorimeter. Emission spectra were recorded by exciting at 318 nm with a scan rate of 500 nm/min, and the excitation and emission slits were fixed at 6 and 18 nm, respectively. Four scans were collected and averaged for each spectrum. The temperature of the quartz cuvette (1.000 cm path) was controlled by recirculating water from a thermostat to the housing at 15, 25, and 35 °C (±0.1 °C). In the experiments with β-CD, the PRF concentration was fixed at 1.57×10^{-5} M, and the ratio β-CD/PRF increased up to 100 fold. A stock solution containing PRF + β-CD was added directly to the cell with a micropipet. This procedure minimizes possible errors due to the manipulation of the cuvette or to slight changes in its position with respect to the radiation source. Typical volumes used were 2 mL in the cell and 1 mL of stock solution added along the concentration range.

UV spectra were recorded with a HP-8452A diode array spectrophotometer, with an integration time of 1 s (10 spectra per record) by using 1.000 cm quartz cells. The temperature was kept constant at 25.0 ± 0.1 °C by recirculation of water from a thermostat.

Molecular Mechanics Study. The software for the molecular mechanics (MM) calculations was *Insight II*,[8] implemented in an IRIS 4D/310VGX workstation of Silicon Graphics. Energy minimization of the isolated host and guest molecules was performed with the *Discover* module, employing the CVFF[9] force field. A sequence of algorithms (steepest descents, conjugate gradients, and Newton−Raphson) was used until the root-mean-squares of the derivatives were below 0.001 kcal Å^{-1}. To account for the solvent effect, a dielectric constant of 80 was included into the Coulombic term of the force field.

The molecular structure of the β-CD, highly regular, with the seven bridge oxygen atoms practically coplanar, has been generated by connecting seven units of α-D-glucopyranose by α-(1-4) linkages. As for PRF, different conformations of the guest have been built with the *Constraint/Rotor* module of the program, by changing the torsion angle between phenyl nuclei at steps of 10° and evaluating the energy at each point.

The inclusion process has been accomplished by rigid docking experiments with the *Docking* module, together with MM minimizations. To include all the possible interactions, cutoff distances of 20 Å were taken into account when evaluating the Coulomb and van der Waals contributions to the energy.

Results and Discussion

Absorption Spectroscopy. The UV spectrum shows three maxima, at 210, 250, and 306 nm, with molar absorptivities of $1.94 \cdot 10^4$, $7.23 \cdot 10^3$, and $3.03 \cdot 10^3$ L mol^{-1} cm^{-1}, respectively.

The addition of β-CD to a $1.00 \cdot 10^{-4}$ M solution of PRF, up to concentrations of 45:1 (β-CD/PRF), reduces the absorbance of the bands at 210 and 250 nm. This observation, together with the presence of three isosbestic points at 220, 234, and 268 nm (Figure 2a) are an indication of the equilibrium corresponding to the complex formation. In Figure 2b, the difference in the absorbance at 250 nm with respect to the propafenone versus the added β-CD is plotted. Assuming additivity, the absorbance can be expressed as the sum of all the contributions. Thus,

$$A = A_{CD} + A_P + A_{P:CD} = \epsilon_{CD}|CD| + \epsilon_P|P| + \epsilon_{P:CD}|P{:}CD| \quad (1)$$

being A the measured absorbance, A_P, and A_{CD} those due to

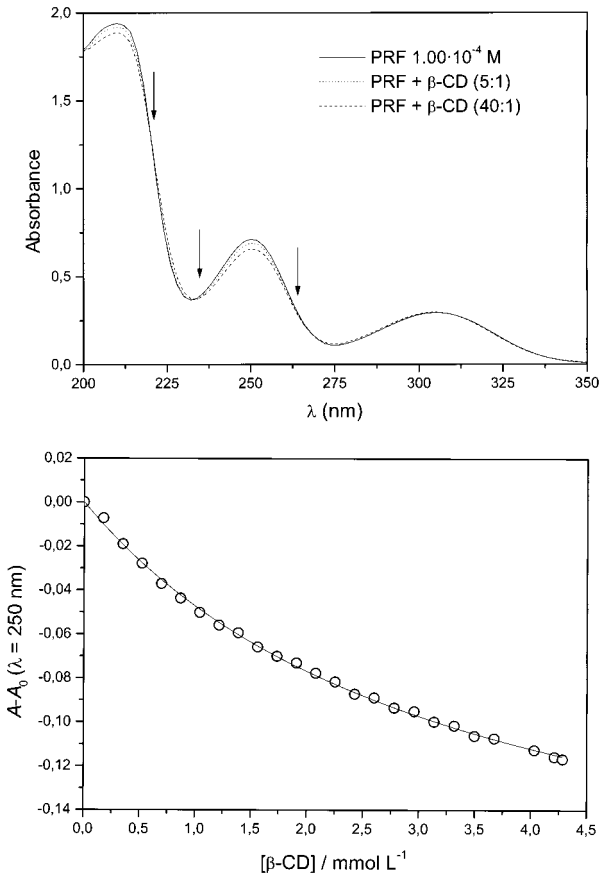

Figure 2. (a) UV spectrum of PRF:β-CD system at several ratios β-CD/PRF. The arrows indicate the isosbestic points. (b) Absorbance at 250 nm as a function of [β-CD].

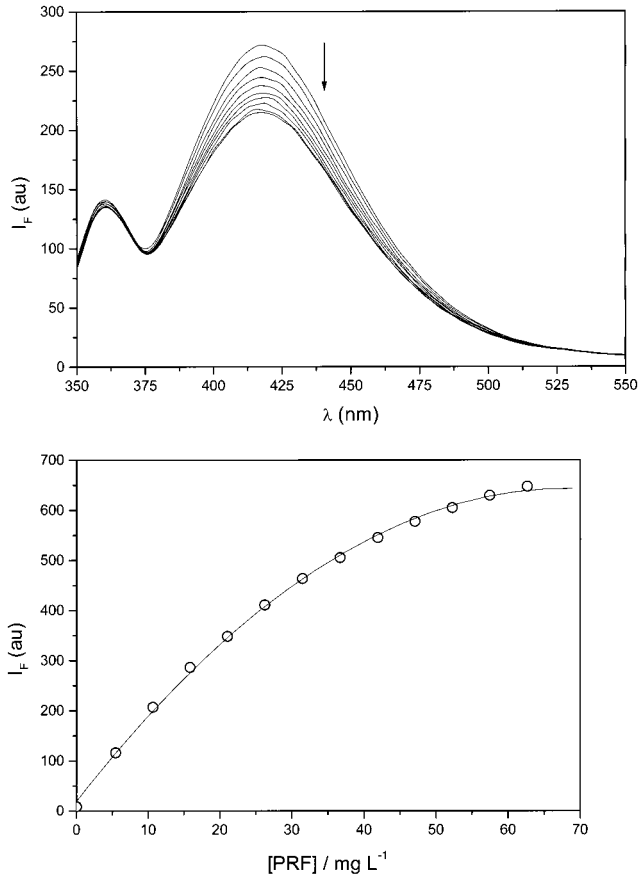

Figure 3. (a) Fluorescence spectra of PRF/β-CD system (λ_{exc}=318 nm). The arrow shows the decrease in fluorescence with [β-CD] ranging from 0 to 1.4 mM. (b) Fluorescence emission of PRF in water at 417 nm and 25 °C (solid line represents the polynomial fit).

PRF and to CD, respectively; $\epsilon_{P:CD}$ is the molar absorptivity of the complex, and ϵ_P and ϵ_{CD}, those of PRF and β-CD, respectively; the terms between bars represent the concentration of each component. The absorption of β-CD ($\epsilon = 15.0 \pm 0.1$ L mol^{-1} cm^{-1} at 250 nm), although negligible when working at long wavelengths and diluted solutions, must be accounted for in this case, since the concentration of β-CD is considerable higher than that of PRF. Otherwise, the first summand in eq 1 vanishes, resulting in a simpler expression. Since the total concentration of PRF and β-CD are the sum of the component in free and complexed form, by rearranging terms we have

$$A = \epsilon_{CD}(CD_0 - |P{:}CD|) + \epsilon_P(P_0 - |P{:}CD|) + \epsilon_{P:CD}|P{:}CD|$$

$$= |P{:}CD|(\epsilon_{P:CD} - \epsilon_P - \epsilon_{CD}) + \epsilon_P P_0 + \epsilon_{CD}CD_0$$

$$\Delta A = A - \epsilon_P P_0 - \epsilon_{CD}CD_0 = A - A_P^0 - A_{CD}^0 = \Delta\epsilon|P{:}CD| \quad (2)$$

an equation that relates the changes in the absorbance to the concentration of the complex, which are a function of the binding constant, K_b. However, the calculation of the binding constants by UV spectroscopy in this system is complicated by several factors. On one hand, there is the meagerness of the changes in ΔA; on the other hand, the low extinction coefficient of PRF makes it compulsory to operate at high concentrations and, consequently, use large amounts of β-CD. In these conditions, the dominant term is that due to the absorption of the free CD over that of the complex. This fact limits the range of the binding isotherm in which one can work, up to $5 \cdot 10^{-3}$ M in β-CD, and it could lead to the underestimation of the binding constant. In Figure 2b, the fit by nonlinear squares fitting (NLSF) to eq 1 is plotted (dashed line). From this fit, the binding constant obtained is 307 ± 9 L mol^{-1}, together with a molar absorptivity for the complex of 2040 ± 40 L mol^{-1} cm^{-1}.

Fluorescence Spectroscopy. Fluorescence spectroscopy is usually more sensitive than absorbance and, provided there are changes in the emission of the substrate, the complex, or both, is a better technique to estimate binding constants and thermodynamic parameters.

For PRF in water, the fluorescence emission reaches a maximum at 417 nm (Figure 3a). In Figure 3b, the maximum in intensity has been plotted versus the concentration at 25 °C, according to the instrumental setup described in the preceding section. The fluorescence can be considered linear up to $3 \cdot 10^{-5}$ mol L^{-1} (ca. 11 ppm, with a slope of 18.7 ± 0.7 ppm^{-1}). The emission intensity can be fitted by least squares to a parabola along the whole range, yielding $I_F = 20 + 18.3c - 0.135c^2$ (where c is expressed as milligram of PRF per liter of solution).

When increasing the temperature, the fluorescence of PRF is considerably reduced. For instance, in the conditions described above, at a PRF concentration of 1.57×10^{-5} M, the emission reduces to 26% of its initial value from 15 to 45 °C. This fact has limited the study to a maximum temperature of 35 °C, to obtain fair values of the binding constant.

The emission of fluorescence for the β-CD + PRF system is shown in Figure 3a. Problems due to the lack of linearity can be avoided working at concentrations below the limit of linearity. Thus, we have fixed the PRF concentration at $1.57 \times$

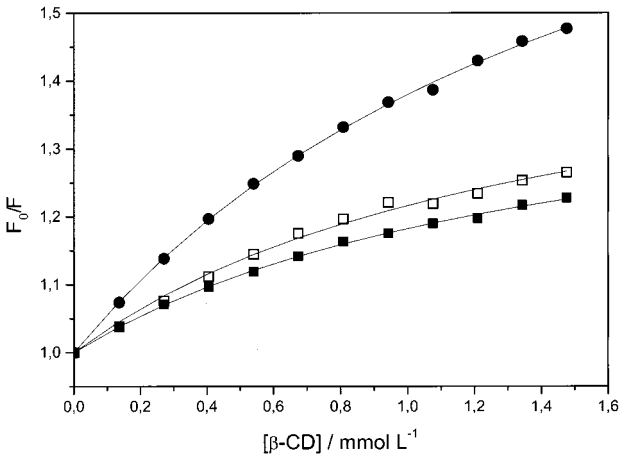

Figure 4. Ratio F_0/F versus [β-CD] at 15 (●), 25 (□), and 35 °C (■), together with the fits to eq 3.

10^{-5} M in all the experiments. A reduction in the fluorescence of PRF when increasing β-CD amount is noticed, although the quenching is not complete and the emission intensity converges to a constant value. This decrease in the intensity has been observed in complexes between CDs and condensed aromatic hydrocarbons and heterocycles, such as acridine,[10] phenazine,[11] or naphthalene derivatives.[12] It is in contrast to the trend displayed by many fluorophores, in which the common behavior is a fluorescence enhancement, a fact which has found analytical applications in increasing the detection limits of some substances of interest (PAHs, drugs,[13] and so on). The specific nature of this quenching, whether static or dynamic, can be deduced by temperature studies or by time-resolved fluorescence measurements.[14] Some of the aforementioned investigations point to a combination of both mechanisms, mainly the static one. This form of quenching removes a fraction of the fluorophores in solution, and in the simplest case, it results from the formation of a dark complex between the fluorophore and the quencher. In our case, the complex is fluorescent, although less emissive than PRF itself.

In Figure 4, the ratio F_0/F versus the concentration of β-CD is plotted for the three temperatures studied. The graphs show the usual tendency observed in static quenching processes, in which increasing T reduces the stability of the complex and raises the concentration of the free fluorophore, thus lowering the ratio F_0/F. The presence of three isosbestic points upon the addition of β-CD, observed in the UV spectrum of PRF, constitutes an additional proof of the type of mechanism. The complex formation affects to the ground state of the fluorophore, and as a result, it gives rise to alterations in the absorption spectrum, whereas collisional quenching only affects the excited states of the fluorophore.

In the general case of a 1:1 equilibrium, in which the complex and the substrate are fluorescent, the increase/decrease in intensity is given by

$$\frac{F_0}{F} = \frac{1 + K_b|\mathrm{CD}|}{1 + aK_b|\mathrm{CD}|} \tag{3}$$

with F_0 being the fluorescence of PRF, F the measured fluorescence at each point, |CD| the concentration of nonbonded β-CD, and K_b the binding constant; a is related to the quantum yields and absorptivities of PRF in its free and complexed form through $a = \epsilon_{cx}\phi_{cx}/\epsilon_{PRF}\phi_{PRF}$. This equation can be linearized to give

$$\frac{1}{F_0/F - 1} = \frac{a}{1 - a} + \frac{1}{(1 - a)K_b|\mathrm{CD}|} \tag{4}$$

Assuming the amount of CD added, CD_0, is much higher than the fixed PRF, |CD| can be replaced by CD_0, and by plotting the ratio F_0/F versus $1/CD_0$, a straight line will be obtained. Thus, the binding constant can be deduced directly from the slope and intercept of the Benesi−Hildebrand plots, by least squares (eq 4). The problem with such type of plots lies in the high statistical weight given to the points having lower concentrations, which results in errors in the slope. This can be overcome by performing a nonlinear least-squares regression analysis based upon eq 3,[15] using the values obtained from eq 4 as initial estimates. The fitted curves are plotted in Figure 4, and the resultant parameters collected in Table 1. The trend with T indicates a higher stability at lower temperatures, as corresponds to an exothermic process. Regarding the parameter a, it increases with temperature. Since the molar absorptivities of the PRF and the complex are nearly the same, and the fluorescence diminishes with T, the quantum yield of the complex PRF:β-CD must decrease more slowly with T than that of PRF.

From the dependence of K_b with the temperature, it is possible to deduce the enthalpy and entropy of the binding process through the van't Hoff equation, provided these magnitudes remain constant within the considered temperature range:

$$R \ln K_b = \Delta S - \frac{\Delta H}{T} \tag{5}$$

By substitution of the binding constants in this expression, the linear fit gives $\Delta H = -6.4 \pm 0.4$ kJ mol^{-1} and $\Delta S = 37 \pm 1$ J K^{-1} mol^{-1}, with a correlation coefficient of 0.998.

1D ^{1}H NMR Spectroscopy. *Binary System.* The well-known spectrum of β-CD is displayed in Figure 5 together with the assignment of its protons.[16] The spectrum of PRF, divided into the aromatic and the aliphatic regions, is plotted in Figure 6. The elucidation of the signals has been accomplished by using conventional procedures in NMR, such as COSY and irradiation on selected resonances. This molecule presents a keto−enol tautomerism, noticed in the vanishing of the resonance of the H_K protons after 24 h, while the H_L transforms in an almost uncoupled signal, as H is exchanged by deuterium on site K (Figure 6). The diastereotopic H_F protons are, together with H_D and H_E, a clear example of an ABCXY type system, although due to the apparent equivalence of the H_F the spin system is reduced to an AB_2XY type.

Ternary System. To propose a model to obtain the stoichiometry and the binding constant, it is necessary to know whether the spectra of the pure components display changes with concentration within the concentration limits under study. No variations in the shape or position of the resonances were observed in PRF−D_2O solutions 30, 76, and 120 mM (solubility limit), within the experimental error. The PRF concentration was kept constant at 4 mM and that of β-CD was varied to obtain different molar ratios R = [PRF]/[β-CD], ranging from 0 (pure β-CD) to ∞ (pure PRF).

Although the spectra undergo significant modifications, mainly due to the overlapping of different resonances when the amount of β-CD varies, we could not detect the appearance of new signals, indicative of a possible enantiomeric resolution. Only the splitting of the signal of the isochronous H_F protons of PRF, which are converted into nonequivalent protons by the CD, was perceived.

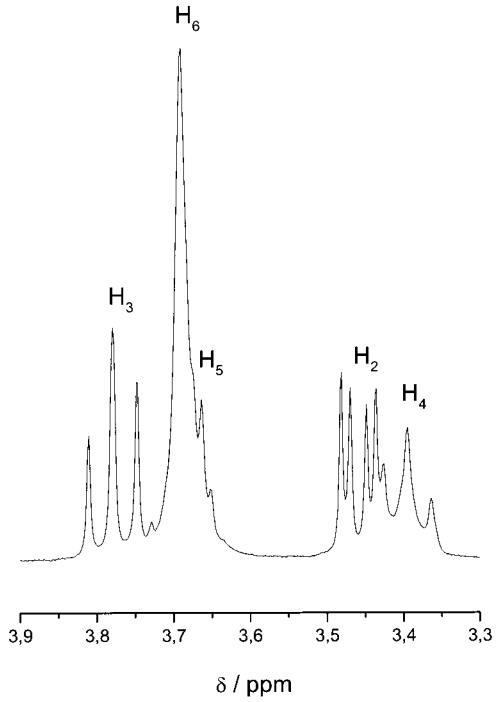

Figure 5. ^{1}H NMR spectrum and proton assignment of β-CD in D_2O.

TABLE 1: Binding Constants for PRF + β-CD in Water at Different Temperatures by Steady-State Fluorescence

T (K)	$K_b \cdot 10^{-3}$ (L/mol)	a
288	1.19 ± 0.04	0.491 ± 0.007
298	1.09 ± 0.11	0.66 ± 0.01
308	1.00 ± 0.04	0.690 ± 0.007

Figure 7 displays the spectra for all the measured molar ratios, although for the sake of clarity only two zones of the complete spectra are shown, corresponding to β-CD and to the aromatic part of PRF. The tracking of the resonances of the different protons, a tedious task in some cases due to overlapping of signals, was accomplished via irradiation of the pertinent resonances. The changes in the chemical shifts with respect to the pure substances in water, $\Delta\delta$, are plotted in Figure 8.

As can be noticed in these figures, the aromatic protons J, M, and N undergo the largest changes (a maximum of ca. 0.26 ppm when the complex is fully formed), shifting to high field. This trend is common for aromatic guests[17] and denotes a microenvironment of high electron density, due to the lone pairs of the seven glycosydic O4 atoms of the β-CD at its equatorial plane. This observation, together with the remarkable upfield shifts shown for the H5 and H3 protons of β-CD (Figure 7) are a clear sign of the inclusion of, at least, one of the aromatic rings of PRF. On the other hand, H1, H2, and H4, protons, located at the outer face of the CD, are scarcely shifted (less than 0.020 ppm).

Assuming an 1:1 stoichiometry and a fast exchange on the NMR time scale,[18] the measured chemical shifts, δ, can be expressed as the sum of the contributions of the chemical shifts due to the complex, $\delta_{P:CD}$, and to the host/guest, δ_i ($i = \beta$-CD or PRF), each one averaged with its molar fraction, that is

$$\delta = \chi_i \delta_i + \chi_{P:CD} \delta_{P:CD} \qquad (6)$$

The binding constant can be estimated by a nonlinear fit of the increments in the chemical shifts, $\Delta\delta$, versus the molar ratio. It should be noticed that the method is essentially the same as the one described in the preceding section for the fluorescence and

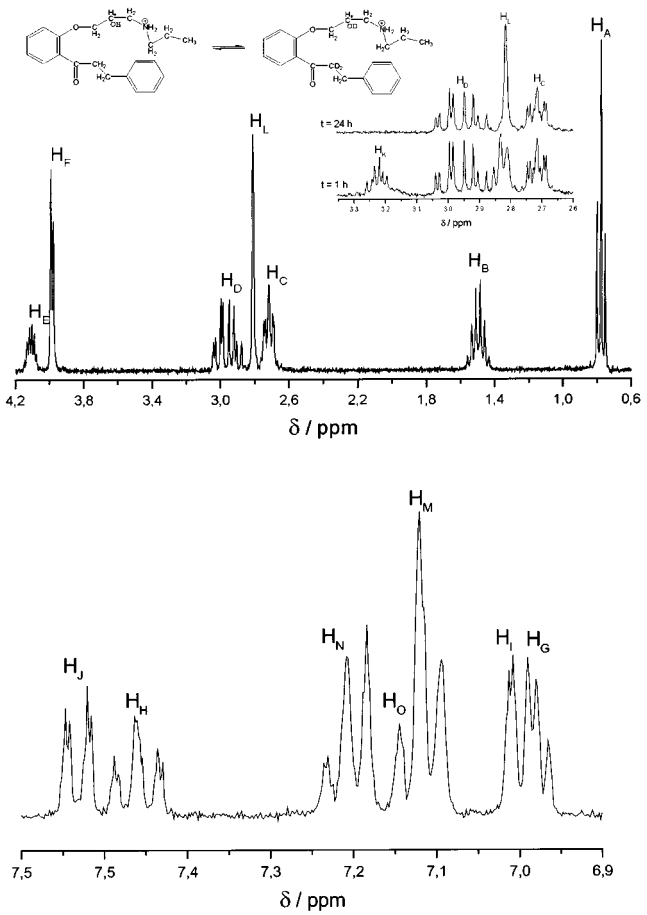

Figure 6. ^{1}H NMR spectrum and proton assignment of PRF in D_2O (aromatic and aliphatic regions).

that it can be applied to protons of either the host or the guest. The solid lines in Figure 8 represent the different fits to eq 6. The binding constants thus obtained with PRF and β-CD protons are listed in Table 2 (only those calculated with the protons that undergo the highest shifts are shown). The calculations with the PRF protons yield virtually the same results as those of fluorescence measurements (average value, weighed according to each error, of $(1.05 \pm 0.15) \cdot 10^3$ L mol^{-1}). Both techniques gave values of K_b higher than that obtained by absorption spectroscopy, as explained in the preceding section. The constants obtained with the protons of the cavity, H5 and H3, are higher than those obtained with the PRF ones (specially H3), although the relative errors are higher (ca. 30%). These high values could indicate a meager contribution of a labile (β-CD)$_2$:PRF complex formed in an excess of CD, an effect that could be manifested only with the β-CD protons and at low relations of PRF/β-CD, since two CDs would be implicated in the complex formation but only one of PRF.

3D Structure of the Complex. ROESY experiments give valuable information regarding dipolar interactions between nuclei of different molecules that are close to each other. It is best suited to systems having molecular weight below 2 kD, such as CD complexes.[17] This technique can be advantageously used together with MM to reproduce the preferred structure or structures of a complex in solution.[19]

Different from many studies of complexes with CDs, in which the guest is a somewhat rigid molecule, PRF has a considerable torsional ability leading to conformers with quite different structures, not all of them equally capable to form the complex.

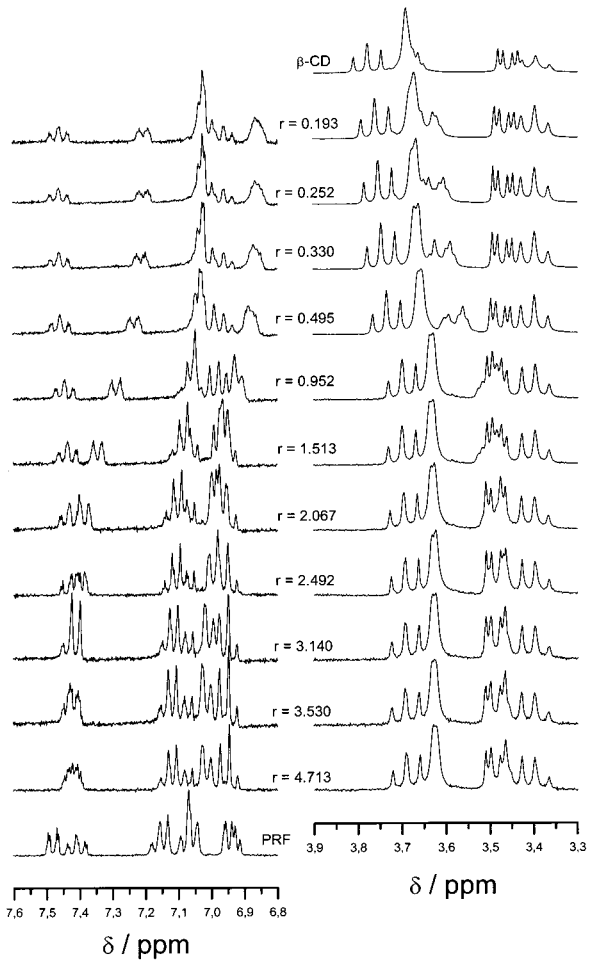

Figure 7. Selected regions of ^1H NMR spectra in D_2O at different ratios [PRF]/[β-CD].

Hence, the first step has consisted in the identification of the 3D structures that PRF can adopt by means of a conformational search.

The most significant degree of freedom observed for this molecule, closely related to the ability of forming a complex with β-CD, is the torsion angle located between the aromatic groups (−Ph−CO−C*H_2−C*H_2−Ph), whose variation leads to three conformers of minimal energy, depicted in Figure 9. Two of them have almost the same energy, with the phenyl groups coarsely facing each other (hereafter, conformer A, 66.35°, 98.05 kcal mol^{-1}; conformer C, 306.23°, 97.93 kcal mol^{-1}). The intermediate situation is represented by the conformer B (176.44°, 99.61 kcal mol^{-1}), with a slightly higher energy and an extended chain where both phenyl groups are opposed. The minimal energies for the three conformers arise at angles deviating from the common torsion values for the staggered and eclipsed conformations, due to the nonbonding repulsive forces induced by the aliphatic tail of the PRF. This fragment has polar substituents, such as the hydroxyl and amine groups, that increase the energy of the system in 14 kcal mol^{-1} with respect to the value it would had if only the aromatic rings were considered.

To investigate the inclusion process with the conformers, two stages have been considered in the calculation. First, a rigid docking from the refined structures of the molecules to select the preferred relative orientation in the inclusion and, second, MM minimization of the β-CD:PRF assembly. Rigid docking strategies permit the calculating of the nonbonding energy between the host and the guest and assist in finding the preferred

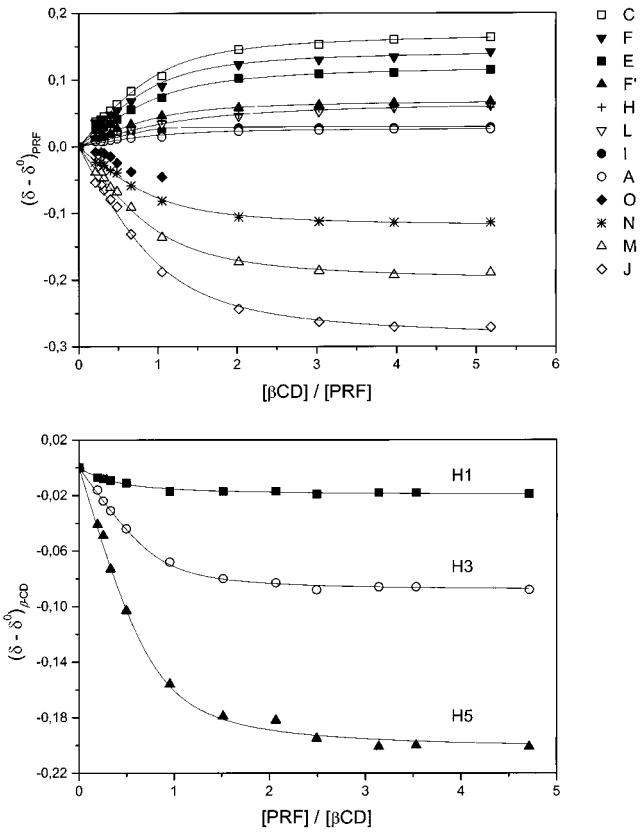

Figure 8. Increments in the chemical shifts versus the molar ratio for selected protons of (a) PRF and (b) β-CD.

TABLE 2: Binding Constants for PRF + β-CD in Water at 22 °C by ^{1}H-NMR

PRF proton	$K_b \cdot 10^{-3}$ (L/mol)	β-CD proton	$K_b \cdot 10^{-3}$ (L/mol)
C	1.2 ± 0.2	H3	2.4 ± 0.6
F	1.13 ± 0.17	H5	1.8 ± 0.5
E	0.96 ± 0.12		
M	1.08 ± 0.16		
J	0.94 ± 0.13		

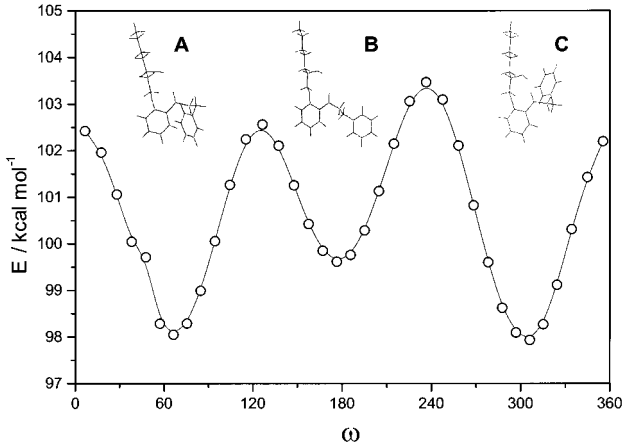

Figure 9. Energy of PRF as a function of the torsion angle −Ph−CO−C*H_2−C*H_2−Ph, and the three conformers of minimal energy: A, B, and C.

orientation of one molecule relative to the other, prior to the minimization. To this purpose, the mass center of β-CD has been chosen as the origin of the Cartesian coordinate system, and a main rotation axis perpendicular to the plane defined by the seven glycosidic O atoms of β-CD. The three PRF conformers are approximated along the rotation axis toward both

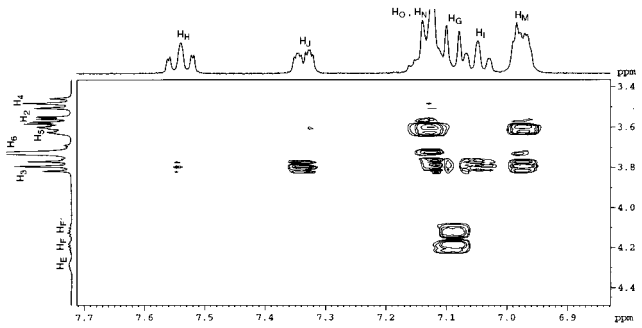

Figure 10. Zoomed view of the ROESY spectrum of the complex (aromatic region of PRF vs β-CD).

TABLE 3: Comparative ROESY Effects for the Complex between β-CD and PRF[a]

		PRF								
		G	H	I	J	L	M	N	O	A
β-CD	**H3**	−	−	++	++	+	++	++	−	+
	H5	−	−	−	−	+	++	++	++	−
	H6	−	−	−	−	−	+	+	+	−

[a] Key: ++ = strong, + = medium, − = none, [β-CD] = 6 mM, and [PRF] = 4 mM.

rims of the β-CD either by the aromatic nucleus or by the aliphatic tail. These approaches give a set of 12 structures, which serve as a starting point for the minimizations. Only a 1:1 stoichiometry has been considered, according to the 1D NMR and fluorescence data.

Among all the possible structures, only some of them are in accordance with the ROESY data (Figure 10). The spectrum shows a clear correlation between some protons of the host and the guest, but particularly between the aromatic ones of PRF and those of the inner part of β-CD (H3, H5) and H6, at the narrower rim. No correlation is observed, however, between the aliphatic chain of PRF with any of the β-CD protons, except a clear NOE (not shown in Figure 10) between the terminal methyl group (A) and the H3. This could indicate a participation of a 2:1 complex that only affects a small part of the PRF. The rest of the chain could remain outside the cavity, although this would modify the values of the binding constant obtained from the shifts of the β-CD protons. In Table 3, the relative NOE effects are collected. Protons I, J, M, and N correlate strongly with H3, and similar behavior is found with M, N, and O with H5, and, to a lesser extent, with H6. The only aliphatic protons that seem to interact are L, that is, those positioned between the two phenyl groups.

TABLE 4: Binding Energies (kcal/mol) for β-CD:PRF Complex

	conformer A	conformer B
E_{bind}	−39.35	−38.97
E_{CD}	104.82	104.82
E_P	98.05	99.61
$E_{P:CD}$	163.52	165.46

All this evidence point to a complex in which the terminal phenyl group must be found at the narrower rim, whereas the other phenyl is at an equatorial position within the β-CD, the aliphatic part remaining outside. It must be noticed that the lack of correlation between the inner protons of β-CD and the aliphatic ones of PRF is compatible with the remarkable changes in the chemical shifts in the 1D spectra (more than 0.1 ppm). This fact could be due to modifications of the molecular geometry and the microenvironment of PRF which do not imply the total inclusion into the cavity: for instance, a small contribution of a 2:1 complex entering a second CD by the terminal methyl group. The fact that the inclusion is not affecting the chiral carbon could explain why no new signals have been observed in the NMR spectrum, indicative of an enantiomeric resolution of the molecule, and the lack in the separation efficiency in the reported CE experiments.[6]

According to the ROESY data, only 2 of the 12 possible structures calculated by MM seem to fulfill these geometric conditions. Both structures are derived from the conformers A and B, the PRF entering the cavity with the phenyls by the wider edge (Figure 11). The C conformer has the phenyl rings in such an extended structure that it cannot fit properly in the CD. The formation energies for the complexes, calculated as $E_{bind} = E_{P:CD} - E_P - E_{CD}$, are compiled in Table 4. As was to be expected, the binding energies are close to each other, suggesting that the formation of the complex is not very sensitive to conformations A or B.

In terms of structure−stability relationships, the PRF:β-CD complex is a rather complicated system, not very suitable to simple interpretations. Nevertheless, the values of the binding constants make sense when they are compared to those of aromatic compounds with β-CD. Aromatic hydrocarbons usually give binding constants ca. 10^2 L mol^{-1}. For example, calorimetric data leads to $K_b = 107$ for benzene;[20] 140 for toluene,[21] whereas for xylenes,[22] K_b ranges from 120 to 230 L mol^{-1}. For

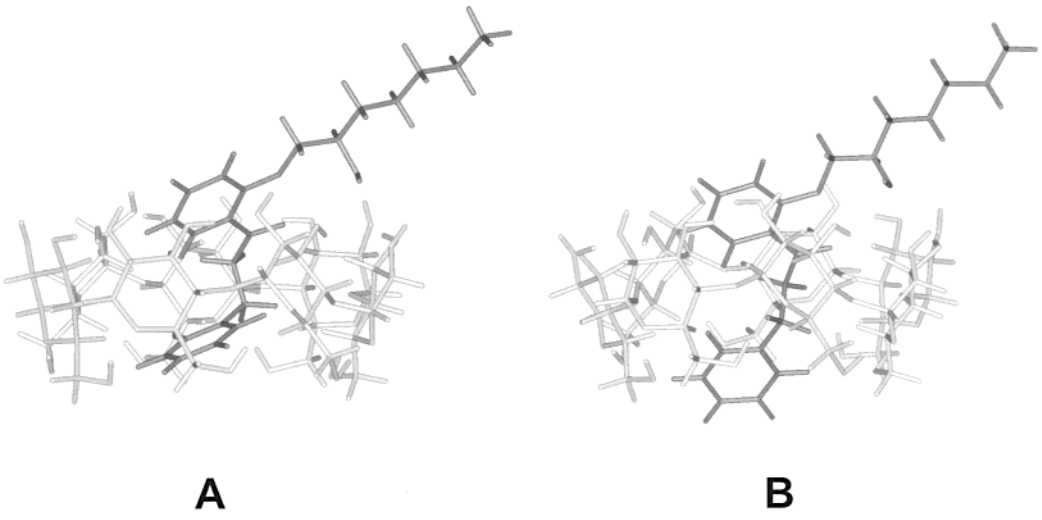

Figure 11. Minimized structures of the complex compatible with ROESY data, as derived from conformations A and B.

PRF, K_b is about 1 order of magnitude higher, which is reasonable considering that two phenyl groups are filling the cavity and, consequently, increasing the hydrophobic surface lodged. The values of the change in the enthalpy and entropy upon binding for benzene are -3.5 kJ mol^{-1} and 8.1 J K^{-1} mol^{-1},[20] respectively, and -1.9 kJ mol^{-1} and 10.8 J K^{-1} mol^{-1},[23] respectively, from vapor pressure measurements. Our values, as estimated from the fluorescence experiments, are $\Delta H = -6.4$ kJ mol^{-1} and $\Delta S = 37 \pm 1$ J K^{-1} mol^{-1}. The exothermicity of the process is the usual in inclusion complexes.[24] As far as the entropy is concerned, the interpretation is not straightforward, since it results from the sum of several contributions of different signs and of a difficult evaluation. On the one hand, it is the entropy loss associated to the binding of the PRF; on the other, the release of water molecules that are inside the cavity and coating the guest. More effects must be taken into account if the substrates possess groups able to form hydrogen bonds. In our case, the change in the entropy is relatively high, and according to the structure of the complex, together with the complete release of the highly structured water of the cavity, two phenyl groups must be dehydrated, which yields a higher entropy for PRF as the guest than for benzene or its derivatives.

Conclusions

The system PRF and PRF + β-CD in aqueous solutions have been studied by absorption and fluorescence spectroscopies, together with 1D and 2D ^{1}H NMR and molecular modeling. The inclusion complex formed has less absorptivity and quantum yield than PRF in water, being that the quenching is mainly static in nature, as deduced from the temperature dependence of the fluorescence emission. The nonlinear fit of the fluorescence data at 15, 25, and 35 °C yields association constants that decrease with the temperature, and changes in enthalpy and entropy upon binding of -6.4 kJ mol^{-1} and 37 J K^{-1}mol^{-1}, respectively. The relatively high value of the entropy follows from the dehydration of both phenyl rings of the PRF in the process, together with the conversion of the water of the β-CD cavity into bulk water. The induced chemical shifts in the 1D ^{1}H NMR experiments confirm a predominantly 1:1 stoichiometry and give values for the binding constant obtained with different protons of PRF that are in excellent agreement with those obtained by fluorescence. ROESY experiments reveal the strongest correlation between the protons H3 and H5 of the cavity and the aromatic ones of the PRF, indicating that the phenyl groups are completely buried into the cavity. No interaction between the aliphatic chain of PRF and the host molecule is observed, except by a small NOE between the terminal methyl group of the chain and the H3 proton, which would suggest a slight participation of a 2:1 stoichiometry in an excess of β-CD. The geometry of the different possible complexes, according to the ROESY data and to molecular modeling experiments, is given, indicating a certain selectivity of the β-CD to the conformation of the PRF.

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References and Notes

(1) (a) Atwood, J. L.; Davies, J. E. D.; Macnicol, D. D.; Vögtle, F. *Comprehensive Supramolecular Chemistry*; Pergamon: Tarrytown, NY, 1996; Vol. 2 (Molecular Recognition: Receptors for Molecular Guests). (b) Gellman, S. H. *Chem. Rev.* **1997**, *97* (5); and thematic issues contained herein.

(2) Atwood, J. L.; Davies, J. E. D.; Macnicol, D. D.; Vögtle, F. In *Comprehensive Supramolecular Chemistry*; Szejtli, J., Osa, T. Eds.; Pergamon: Tarritown, NY, 1996; Vol. 3 (Cyclodextrins).

(3) (a) Connors K. A. *Chem. Rev.* **1997**, *97*, 1325. (b) González-Gaitano, G.; Ortega, F.; Guerrero-Martínez, A.; Tardajos G. *Langmuir* **2001**, *17*, 1392. (c) González-Gaitano, G.; Crespo, A.; Tardajos, G. *J. Phys. Chem.* **2000**, *104* (8), 1869.

(4) (a) Janousek, P. T. *Pediatr. Cardiol*. **1994**, *15*, 190. (b) Bryson, H. M.; Palmer, K. J.; Langtry, H. D.; Fitton, A. *Drugs* **1993**, *45*, 85. (c) Capucci, A.; Villani, G. Q.; Aschieri, D.; Piepoli, M. *Intern. J. Cardiol*. **1999**, *68*, 187.

(5) (a) Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045. (b) Duchene, D. *Cyclodextrins and Their Industrial Uses*; Editions de Santé: Paris, 1987. (c) Frömming, K. H.; Szejtli, J. *Cyclodextrins in Pharmacy*; Kluwer Academic: Dordrecht, 1994.

(6) Guanbin, L.; Xiuli, L.; Chenfu, Z.; Aiyou, H.; Yafen, G. *Anal. Chim. Acta* **2000**, *421*, 27.

(7) Vespalec, R.; Bocek, P. *Chem. Rev.* **2000**, *100*, 3715.

(8) *Insight II*, ver. 3.0.0; Biosym Technologies: San Diego, 1995.

(9) Dauber-Osguthorpe, P.; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J. *Proteins: Struct., Funct., Genet.* **1988**, *4* (1), 31.

(10) Schuette, J. M.; Thilivali, N.; Muñoz de la Peña, A.; Greene, K. L.; Williamson, C. K.; Warner, I. M. *J. Phys. Chem.* **1991**, *95*, 4897.

(11) Schuette, J. M.; Thilivali, N.; Warner, I. M. *J. Phys. Chem.* **1992**, *96*, 5309.

(12) Fraiji, E. K.; Cregan T. R.; Werner, T. C. *Appl. Spectrosc.* **1994**, *48* (1), 79.

(13) Hinze, W. L.; Dai, F.; Frankewich, R. P.; Thimmaiah; Szejtli, J. In *Comprehensive Molecular Chemistry*; Szejtli, J., Osa, T., Eds.; Pergamon: Tarrytown, NY, 1996; Vol. 3 (Cyclodextrins), p 588.

(14) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy;* Kluwer Academic/Plenum Publishers: NY, 1999; pp 238−249.

(15) Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987; pp 339.

(16) Demarco, P. V.; Thakkar, A. L. *J. Chem. Soc. (D)* **1970**, 2.

(17) Scheneider, H. J.; Hacket, F.; Rüdger, V. *Chem. Rev.* **1998**, *98*, 1755.

(18) Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987; p 189.

(19) Lipkowitz, K. B. *Chem. Rev.* **1998**, *98*, 1829.

(20) Gómez-Orellana, I.; Hallén, D. *Thermochim. Acta* **1993**, *221*, 183.

(21) Sanemasa, I.; Akamine, Y.; *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2059.

(22) Poh, B. L.; Chow, Y. M. *J. Inclusion Phenom.* **1992**, *14*, 85.

(23) Tucker, E. E.; Christian, S. D. *J. Am. Chem. Soc.* **1984**, *106*, 1942.

(24) Rekharsky, M.; Yoshihisa, I. *Chem. Rev.* **1998**, *98*, 1875.