# Complexation of Adamantyl Compounds by β-Cyclodextrin and Monoaminoderivatives

**Jorge Carrazana, Aida Jover, Francisco Meijide, Victor H. Soto, and José Vázquez Tato***

*Departamento de Química Física, Facultade de Ciencias, Universidade de Santiago de Compostela, Campus de Lugo, Avda. Alfonso X El Sabio, s/n, 27002 Lugo, Spain*

*Received: February 2, 2005; In Final Form: March 10, 2005*

Since the β-cyclodextrin cavity is not a smooth cone but has constrictions in the neighborhoods of the H3 and H5 atoms, the hypothesis that bulky hydrophobic guests can form two isomeric inclusion complexes (one of them, $c_p$, is formed by the entrance of the guest by the primary side of the cavity, and the other one, $c_s$, results from the entrance by the secondary side) is checked. Thus, the inclusion processes of two 1-substituted adamantyl derivatives (rimantidine and adamantylmethanol) with β-cyclodextrin and its two monoamino derivatives at positions 6 (6-$NH_2$β-CD) and 3 (3-$NH_2$β-CD) were studied. From rotating-frame Overhauser enhancement spectroscopy experiments, it was deduced that both guests form $c_s$ complexes with β-CD and 6-$NH_2$β-CD but $c_p$ complexes with 3-$NH_2$β-CD. In all cases, the hydrophilic group attached to the adamantyl residue protrudes toward the bulk solvent outside the cyclodextrin cavity. The thermodynamic parameters (free energy, equilibrium constant, enthalpy, and entropy) associated with the inclusion phenomena were measured by isothermal titration calorimetry experiments. From these results, the difference in the free energy for the formation of the two complexes, $c_s$ and $c_p$, for the same host/guest system has been estimated as being $11.5 \pm 0.8$ kJ mol$^{-1}$. This large difference explains why under normal experimental conditions only one of the two complexes ($c_s$) is detected. It is also concluded that a hyperboloid of revolution can be a better schematic picture to represent the actual geometry of the cyclodextrin cavities than the usual smooth cone or trapezium.

## Introduction

Natural cyclodextrins are cyclic oligomers built up from 6, 7, or 8 glucopyranose units (named α-, β-, and γ-CD, respectively) linked by α-(1−4)-glycosidic bonds.[1] As a consequence of the $^4C_1$ conformation of the glucopyranose units, all secondary hydroxyl groups are situated on one of the two edges of the ring, and all primary ones are placed on the other edge. They have been used as enzyme models and artificial enzymes[2−5] and have many practical applications in pharmacy and engineering[1] as they can form inclusion compounds by entrapping organic compounds (guests) in the hydrophobic cavity of the cyclodextrin (host). Their structure is very well-known.[6] For instance, the macrocycle has a rigid structure primarily due to the formation of intramolecular hydrogen bonds between the O2−H and O3−H hydroxyl groups of adjacent glucose units, and all of the O4 atoms are virtually coplanar, with less than 0.25 Å deviation from the common mean plane.[6−8] It is frequently characterized as a doughnut or wreath-shaped truncated cone (Figure 1), the larger diameter corresponding to the secondary rim. The interior of the cavity is not a smooth cone but has constrictions in the neighborhoods of the H3 and H5 atoms since these atoms of each glucose residue protrude into the β-CD cavity, forming constrictions to the entrance of guest molecules, the radii of free space (available for guests) being approximately 3.3 and 3.0 Å, respectively.[6,8] Thus, the central region of β-CD can be considered as a *frontier* dividing the β-CD cavity into two different regions, one close to the primary rim and the other one close to the secondary rim.

* Author to whom correspondence should be addressed. Phone: 34-982-28 58 71, ext 24082. Fax: 34-982-28 58 72. E-mail: jvtato@lugo.usc.es; jvtato@yahoo.com.

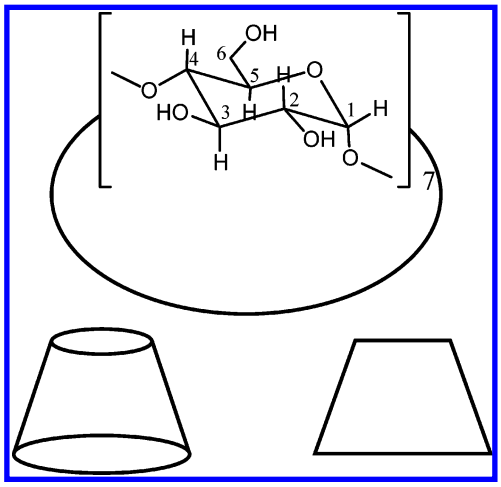



**Figure 1.** Schematic structure of β-CD.

The complexation of adamantane derivatives with β-CD has been the subject of numerous studies.[9−25] Adamantane derivatives form unusually strong complexes with β-CD since the equilibrium constants are the strongest ($\Delta G°$ is usually close to −30 kJ mol$^{-1}$). This results because the adamantyl residue perfectly fits inside the β-CD cavity and water molecules do not have room inside the cavity.[8,22] This residue almost has a spherical shape with a radius of 3.6 Å (estimated from the adamantyl residue of the mono-6-deoxy-6-adamantylamide-β-CD),[22] which is only slightly larger than the radii of free space inside the cavity available for guests in the neighborhoods of the H3 and H5 atoms. In general, the values published for enthalpy, entropy, and the equilibrium constant are in good agreement, but in some cases strong discrepancies can be found

10.1021/jp0505781 CCC: $30.25 
Published on Web 04/19/2005

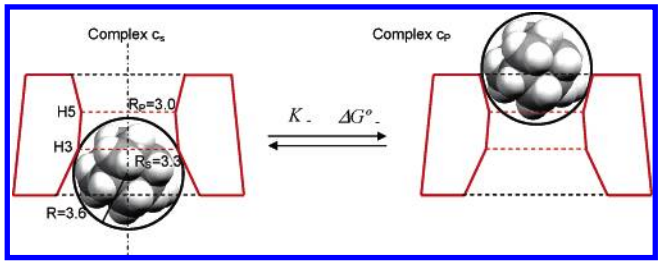



**Figure 2.** Equilibrium between expected inclusion complexes formed by $\beta$-CD and adamantane derivatives, data in Å.

as, for instance, for the complexation of rimantidine with $\beta$-CD (see below). In some studies, the pH was kept constant at values close to the p$K_a$ of the guest, and therefore several species are probably involved in the complexation process, i.e., the thermodynamic parameters obtained are in fact average values of several microscopic processes. The buffer can also interact with the cyclodextrin,[13] influencing in an unknown quantity the values of the thermodynamic parameters. Palepu and Reinsborough[19] have concluded that the charge on the polar group plays a minor role in the host/guest binding (which would largely be dictated by the closeness of fit of the adamantyl moiety within the $\beta$-CD cavity) since, among other reasons, the strongest binding occurred when the charge was neutralized by protonation or deprotonation. In some cases, the structure of the complex is determined from rotating-frame Overhauser enhancement spectroscopy (ROESY) experiments,[22] and the nature of the driving forces for the complex formation have been discussed in terms of van der Waals interactions, hydrogen bonding, hydrophobic interactions, steric strains, release of high-energy water molecules from the cavity, etc.

The proximity of the radii values shown above for $\beta$-CD and the adamantyl residue (the difference being around 10%) suggests that two different isomeric complexes with adamantyl derivatives could be a priori formed, the guest being located either at the primary rim region ($c_p$ complex) or at the secondary rim region ($c_s$ complex; Figure 2). As far as we know, the formation of these kinds of isomeric complexes has not been reported for cyclodextrins. The reason is probably related to the fact that if the guest prefers one of the two locations, then the less stable complex (from a thermodynamic point of view) will be masked by the more stable one. For instance, if the difference in the free energy for the formation of the two complexes is 4$RT$ (10 kJ mol$^{-1}$ at room temperature), then generally only one complex would be detected since it would be in a much higher concentration than the other one (approximately 100:1). For adamantyl guests, if the tendency to optimize the reciprocal concurrence of lipophilic as well as hydrophilic domains at the guest−host interface is decisive in stabilizing the complex,[26] then it is expected that the equilibrium constant for the formation of $c_s$ will be higher than that for $c_p$. Under this condition, only the hindrance of the secondary entrance to the cavity will allow the formation of a $c_p$ complex in high yields. This can be done by substituting one 3-hydroxy group of the secondary rim with an ammonium group since it protrudes toward the center of the rim. Consequently, the 3-deoxy-3-amino derivative, 3-$NH_2$-$\beta$-CD, was synthesized. To study the possible effect of the charge in the host species on the equilibrium constant, the 6-deoxy-6-amino derivative, 6-$NH_2$-$\beta$-CD, was also obtained. If the protonated amino group in the 6-position has a negligible effect on the complexation of a guest entering by the secondary rim in comparison with its complexation by neutral $\beta$-CD, then it is expected that the amino group at the 3-position will not have an effect on the complexation of a guest when entering by the primary rim. Somehow, the methodology is reminiscent of the well-known method of determining the microscopic dissociation constants of amino acids and other biomolecules, and therefore the present system can be used as a model for the binding of a small ligand (drug) to a macromolecule (protein, enzyme, etc.) with two different but not simultaneous binding positions (i.e., the 1:2 stoichiometry complex cannot be formed). Rimantidine and adamantylmethanol ($AdCH_2OH$), 1-adamantyl derivatives, have been chosen as guests.

## Thermodynamic Background

The formation of two isomeric complexes with a 1:1 stoichiometry from a guest, g, and a host, h, is given in eqs 1 and 2

$$h + g \rightleftharpoons c_p \quad K_p;\ \Delta G^\circ_p \tag{1}$$

$$h + g \rightleftharpoons c_s \quad K_s;\ \Delta G^\circ_s \tag{2}$$

p and s meaning that the guest is located at the primary or secondary side of the $\beta$-CD, respectively. From previous equations, eqs 3 and 4 are deduced.

$$2h + 2g \rightleftharpoons c_p + c_s \quad K_+ = K_p K_s \quad \Delta G^\circ_+ = \Delta G^\circ_p + \Delta G^\circ_s \tag{3}$$

$$c_s \rightleftharpoons c_p \quad K_- = K_p/K_s \quad \Delta G^\circ_- = \Delta G^\circ_p - \Delta G^\circ_s \tag{4}$$

The equilibrium in eq 4 is also shown in Figure 2. Obviously, $\Delta G^\circ_-$ corresponds to the free energy for the interchange of the guest between the two location sites of the host.

Two limit situations can be observed. First, if $\Delta G^\circ_p = \Delta G^\circ_s$ (or they are very close to each other), then the concentrations of the two complexes will be equal (or very similar) to each other, and eq 3 would be indistinguishable from the formation of only one complex. Second, if $\Delta G^\circ_p$ and $\Delta G^\circ_s$ are very different, then only one of the two complexes would be detectable since it would be in a much higher concentration than the other one (see example in the Introduction). The previous elemental analysis suggests that, on most occasions, the determination of $\Delta G^\circ_-$ and $K_-$ cannot be undertaken by direct methods.

To determine $\Delta G^\circ_-$, two (or more) different hosts, h″ and h′, leading to the formation of only primary, $c''_p$, or secondary, $c'_s$, complexes (eqs 5 and 6, respectively) can be used.

$$h'' + g \rightleftharpoons c''_p \quad K''_p = \frac{[c''_p]}{[h'']\,[g]} \quad \Delta G''^\circ_p = -RT \ln K''_p \tag{5}$$

$$h' + g \rightleftharpoons c'_s \quad K'_s = \frac{[c'_s]}{[h']\,[g]} \quad \Delta G'^\circ_s = -RT \ln K'_s \tag{6}$$

The hosts have to be chosen in such a way that the structure and thermodynamic parameters associated with $c'_s$ and $c''_p$ are (ideally) identical to those of $c_s$ and $c_p$, respectively. That is to say

$$\Delta G^\circ_p = \Delta G''^\circ_p \quad K_p = K''_p \tag{7}$$

$$\Delta G^\circ_s = \Delta G'^\circ_s \quad K_s = K'_s \tag{8}$$

Under these conditions, eqs 2 and 6 lead to eq 9 with a free energy value equal to zero.

$$h' + c_s \rightleftharpoons c'_s + h \quad K_9 = K'_s/K_s \approx 1$$
$$\Delta G^\circ_9 = \Delta G'_s - \Delta G_s \approx 0 \quad (9)$$

Analogously, eqs 1 and 5 give eq 10.

$$h'' + c_p \rightleftharpoons c''_p + h \quad K_{10} = K''_p/K_p \approx 1$$
$$\Delta G^\circ_{10} = \Delta G''^\circ_p - \Delta G^\circ_{p_s} \approx 0 \quad (10)$$

Equations 5 and 6 give eq 11

$$h'' + c'_s \rightleftharpoons c''_p + h' \quad (11)$$

the free energy being equal to $\Delta G^\circ_- = \Delta G''^\circ_p - \Delta G'^\circ_s$. Thus, the determination of the thermodynamic parameters associated with the reactions in eqs 5 and 6, which can be measured independently, allows the determination of $\Delta G^\circ_-$. Obviously, $\Delta G^\circ_-$ can also be obtained from eqs 2 and 5 (leading to eq 12) since $\Delta G^\circ_s$ is experimentally available

$$h'' + c_s \rightleftharpoons c''_p + h \quad \Delta G^\circ_- = \Delta G''^\circ_p - \Delta G^\circ_s \quad (12)$$

Analogous equations can be written for a second guest, g*, which forms complexes $c^*_s$ and $c'_s$* with h and h′, respectively. It is straightforward to predict that the free energy associated with eq 13

$$c^*_s + c'_s \rightleftharpoons c_s + c'_s* \quad (13)$$

will be equal to zero, i.e., $\Delta G^\circ_{13} \approx 0$. Obtaining $\Delta G^\circ_{13}$ is necessary to measure four independent complexation reactions (analogous to the reactions of eq 2 or 6). Equation 13 can be used as a checking of the validity of the hypothesis in eq 8 and the correctness of the two (h and h′) chosen hosts. However, only indirect evidence can be argued for the validity of eq 7 since eq 1 is not known (accepting that $K_s \gg K_p$).

## Experimental Section

Adamantane derivatives (99%, Aldrich) were used without further purification. $\beta$-CD (kindly supplied by Roquette) was recrystallized twice from distilled water and dried in a vacuum oven. Stock solutions of the reactants were prepared in phosphate buffer 0.05 M from sodium monophosphate and sodium hydroxide at pH = 7.00 ± 0.01 or pH = 5.50 ± 0.01. At these pH values, the amino groups of the hosts and guests are fully protonated. The phosphate buffer used to maintain the pH constant does not interact with cyclodextrins.[27]

The synthesis of 6-$NH_2$$\beta$-CD has been described previously.[28] The 3-$NH_2$$\beta$-CD was synthesized according to a two-step procedure. (i) For 2-deoxy-2-tosyl-$\beta$-cyclodextrin, 0.070 g (2.9 mmol) of NaH was added to a solution of $\beta$-CD (2.87 g, 2.53 mmol) in 40 mL of dry dimethylformamide (DMF). The mixture was stirred at 25 °C overnight. The solution was added with stirring over a mixture of *p*-toluensulfonyl chloride (0.48 g, 2.53 mmol) in 5 mL of dry DMF. The final solution was stirred for 30 min, and DMF was removed in vacuo. The residue was precipitated from acetone (0.2 L), filtered, and dried. The product was used with any further purification. (ii) For 3-deoxy-3-amino-$\beta$-cyclodextrin, 2-deoxy-2-tosyl-$\beta$-cyclodextrin (1 g, 0.78 mmol) was disolved in 25 mL of aqueous ammonia 25%. The solution was stirred at 50 °C for 12 h. After removal of the solvent in vacuo, the residue was precipitated from acetone (0.2 L) and filtered. The product was purified on a C25 sephadex column with an overall yield of 7%.

For the purposes of this paper, structural studies as well as the determination of the thermodynamic parameters associated to the complexation process are required. Isothermal titration calorimetry (ITC)[29] has been largely used in studying complexation processes by cyclodextrins since it allows the determination of enthalpy, entropy, free energy (and therefore the equilibrium constant), and the stoichiometry of the complex in a single experiment. Many examples can be found in the cyclodextrin literature illustrating the usefulness of ordinary $^1$H and $^{13}$C NMR spectroscopy and ROESY for the determination of the structure of complexes in aqueous solutions. For instance, from the analysis of ROESY spectra, Kano et al.[30] have deduced the side by which an anion guest penetrates into the cavity of protonated aminocyclodextrins and the orientation/location of the guest inside the cavity.

The description of the thermodynamic background for ITC experiments can be found elsewhere.[29] Enthalpies of dilution of the hosts and guests were determined in separate experiments. ITC experiments were carried out in a MicroCalc calorimeter at 30.00 ± 0.01 °C. Experimental titration curves were analyzed with the MCS Origin ITC 5.0 program. Average values of the thermodynamic parameters and their standard deviations were calculated from 4 to 6 experimental runs.

For NMR experiments, $D_2O$ (99.90%) was supplied by SDS (France). Acidity was adjusted with KOD (Aldrich, 40% in $D_2O$) to final pD values in the range 7−7.5. Samples were prepared directly in the NMR tubes. Spectra were recorded using a Bruker AMX-500 NMR or a Varian Inova 750 spectrometer operating at 500 MHz for $^1$H and 125 MHz for $^{13}$C. Tetramethylsilane was used as an external reference. $^{13}$C experiments were recorded on a Bruker AC-300 NMR spectrometer operating at 75 MHz and 293.1 K. Conditions for ROESY were as follows: relaxation delay, 0 s; mixing time, 300 ms; spectral width, 10 ppm with 1024 complex points in *f*2; 128 *t*1 values and 8 scans per *t*1 value; $T$ = 298.1 K.

## Results and Discussion

First, it is necessary to distinguish those complexes that have the guests located at the primary side of the cyclodextrin cavity from those that have the guest located at its secondary side. For this purpose, ROESY and $^1$H NMR experiments were carried out. As an example, we will analyze the results obtained for the complexation of rimantidine with the three hosts. All systems obey the fast exchange regime NMR time scale, and therefore the addition of the guest to the cyclodextrin causes up- or downfield shifts of the host and guest protons.

Figure 3 shows the cross peaks observed from ROESY experiments for an equimolar solution of rimantidine and $\beta$-CD. The $^1$H NMR spectrum of $\beta$-CD is very well-known, and it does not require further comments.[31] The assignment of signals of rimantidine in the $^1$H NMR spectrum is simple, and therefore the identification of the cross peaks in the ROE spectrum can be done in a straightforward fashion. It can be observed that H3 and H5 move toward lower chemical shifts, which strongly suggest the formation of a $c_s$ inclusion complex. The ROE spectrum confirms this conclusion since interactions of H5 with Pb and Pc (strong), H3 with Pc, Pb, Pa, and Pe (strong), and a H3−Pd (weak) are observed. Thus, the ROE spectrum clearly indicates that the rimantidinium cation penetrates into the $\beta$-CD cavity by its secondary hydroxyl rim, forming a $c_s$-type complex (see also the schematic structure in Table 1). The structure of this complex is in agreement with previous observations that the adamantyl residue perfectly fits inside the $\beta$-CD cavity (Figure 2, left-hand side).[32] The absence of cross peaks corresponding to the interaction of H5 with Pa, Pb, and Pe suggests that the protonated amino group of rimantidine

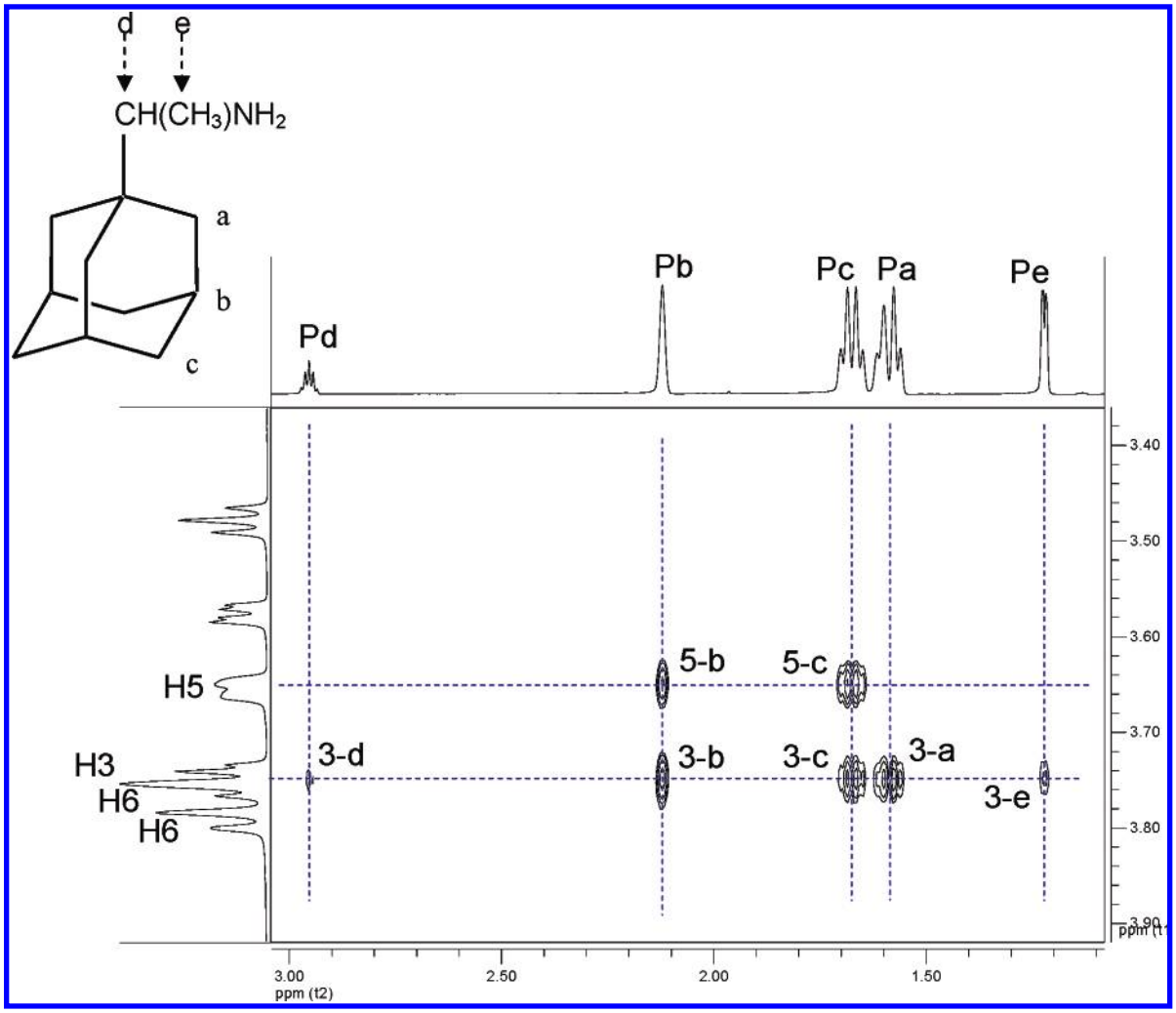
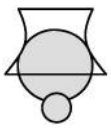
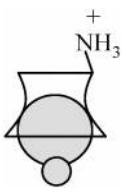
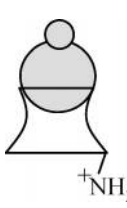



**Figure 3.** Significant cross peaks from the ROE spectrum of the rimantidine/$\beta$-CD system.

**TABLE 1: Thermodynamic Parameters Deduced from ITC Experiments for the Different Host−Guest Systems Studied Here**[a]

| host | thermodynamic parameters | rimantidine | structure | adamantyl-methanol |
|---|---|---|---|---|
| $\beta$-CD | $K_s$ | $(3.52 \pm 0.12) \times 10^4$ |  | $(1.42 \pm 0.05) \times 10^5$ |
| | $\Delta G_s^\circ$/kJ mol$^{-1}$ | $-26.37 \pm 0.08$ | | $-29.89 \pm 0.08$ |
| | $\Delta H_s^\circ$/kJ mol$^{-1}$ | $-28.60 \pm 0.16$ | | $-31.30 \pm 0.14$ |
| | $\Delta S_s^\circ$/J mol$^{-1}$ K$^{-1}$ | $-7.24 \pm 0.60$ | | $-4.33 \pm 0.53$ |
| 6-NH$_2$$\beta$-CD | $K'_s$ | $(1.93 \pm 0.07) \times 10^4$ |  | $(1.02 \pm 0.04) \times 10^5$ |
| | $\Delta G'^\circ_s$/kJ mol$^{-1}$ | $-24.86 \pm 0.09$ | | $-29.05 \pm 0.11$ |
| | $\Delta H'^\circ_s$/kJ mol$^{-1}$ | $-25.02 \pm 0.13$ | | $-30.29 \pm 0.21$ |
| | $\Delta S'^\circ_s$/J mol$^{-1}$ K$^{-1}$ | $-0.46 \pm 0.50$ | | $-4.02 \pm 0.77$ |
| 3-NH$_2$$\beta$-CD | $K''_p$ | $(2.41 \pm 0.61) \times 10^2$ |  | $(1.38 \pm 0.04) \times 10^3$ |
| | $\Delta G''^\circ_s$/kJ mol$^{-1}$ | $-13.82 \pm 0.06$ | | $-18.20 \pm 0.08$ |
| | $\Delta H''^\circ_s$/kJ mol$^{-1}$ | $-17.29 \pm 0.18$ | | $-18.51 \pm 0.02$ |
| | $\Delta S''^\circ_s$/J mol$^{-1}$ K$^{-1}$ | $-11.72 \pm 0.65$ | | $-0.6 \pm 0.7$ |

[a] The temperature was 30.1 °C. The pH was kept constant by using a $NaH_2PO_4/Na_2HPO_4$ buffer (total concentration 0.05 M).

protrudes from the cavity lying in the aqueous bulk solution. Finally, the interaction H3−Pe indicates that the methyl group does not penetrate inside the cavity and lies close to the secondary hydroxy rim.[8] Thus, the complexation probably requires a minor dehydration of the ammonium group, and the clathrated water molecules surrounding the adamantyl group would recover their normal structure in the bulk aqueous solution during the complexation process. Since we have only observed

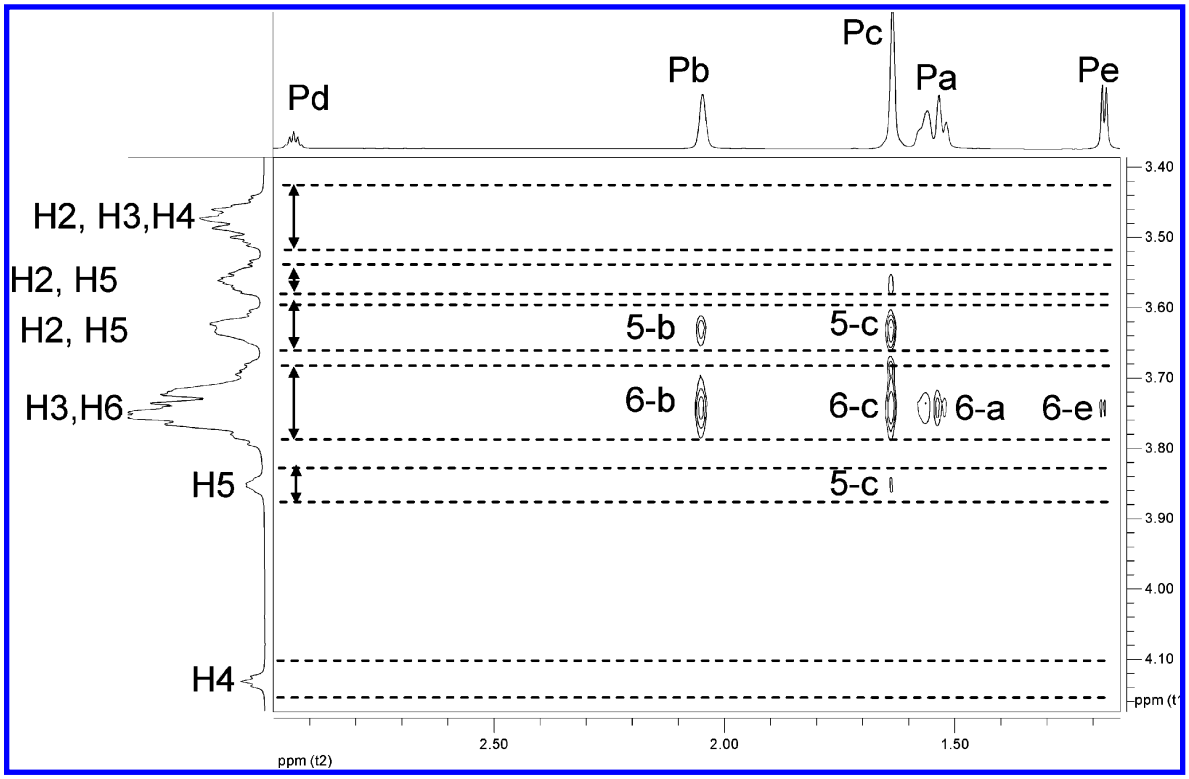



**Figure 4.** Significant cross peaks from the ROE spectrum of the rimantidine/3-$NH_2$-$\beta$-CD system.

the $c_s$ complex, this is a first evidence that $K_- \ll 1$, as predicted above.

The substitution of the hydroxyl group by the amino group breaks the symmetry of $\beta$-CD. As a consequence, each hydrogen atom (H*i*, $i = 1-7$) within each glucose residue of the modified cyclodextrin is not magnetically equivalent, leading to a complex spectrum. Schneider et al.[31] have discussed this topic. The assignment usually starts from $^1$H resonances for the anomeric protons. Although the different types of protons appear at very close $\delta$ (ppm) values, the ROESY spectrum can be solved (Figure 4). Since H2 and H4 protrude toward the solvent (outside the cavity), they cannot interact with any proton of the guest. Therefore, the cross peaks observed for the group of signals at $\delta = 3.54-3.58$ ppm with Pc correspond to the H5−Pc interactions. The same argument applies for the assignment of cross peaks appearing at $\delta = 3.60-3.66$ ppm to the interactions of H5 with Pb and Pc and those at $\delta = 3.68-3.78$ ppm to the interactions of H6 with Pa, Pb, Pc, and Pe. It must be noted that none of the signals appearing at the intervals $\delta = 3.42-3.52$ ppm (six H4, one H2, and one H3) and 4.10−4.16 ppm (one H4) exhibit cross peaks with rimantidine protons. Finally, the assignment of the cross peak at $\delta = 3.83-3.87$ ppm to the H5−Pc interaction is straightforward. The absence of interactions between any of the adamantyl and methyl protons with the H3 protons of the seven glucose residues is remarkable. This shows that these residues have not entered the cavity of the cyclodextrin by its secondary hydroxy rim and that the penetration by the other side is not deep enough to reach H3 protons. Thus, the ammonium group located at the secondary rim blocks out this gate of the cyclodextrin cavity in a very effective way. The conclusion is that a primary $c_p''$ complex (3-$NH_2$-$\beta$-CD = h″) has been formed with the methyl group of the side chain (and consequently the ammonium group) protruding toward the bulk solvent.

The ROE spectrum of the rimantidine/6-$NH_2$-$\beta$-CD system was solved in a similar way. Figure 5 shows the observed

**TABLE 2: Thermodynamic Parameters Deduced from Values of Table 1 for the Reaction in Eq 9**

| guest | $\Delta H_9^\circ$ (kJ mol$^{-1}$) | $T_{303}\Delta S_9^\circ$ (kJ mol$^{-1}$) | $\Delta G_9^\circ$ (kJ mol$^{-1}$) |
|---|---|---|---|
| adamantylmethanol | $1.01 \pm 0.25$ | $0.09 \pm 0.28$ | $0.89 \pm 0.14$ |
| rimantidine | $3.58 \pm 0.21$ | $2.05 \pm 0.23$ | $1.51 \pm 0.12$ |

interactions between guest and host protons. Now the conclusion is that the complex obtained is of the secondary $c_s'$ type (6-$NH_2$-$\beta$-CD = h′) with a structure quite similar to the complex $c_s$ for the rimantine/$\beta$-CD system (see figures in Table 1).

Ad$CH_2OH$/$\beta$-CD (and derivatives) systems were similarly solved. The schematic structures are shown in Table 1. As a conclusion, both adamantylmethanol and rimantidine form $c_s$ inclusion complexes with $\beta$-CD and 6-$NH_2$-$\beta$-CD and $c_p$ inclusion complexes with 3-$NH_2$-$\beta$-CD in agreement with the predictions outlined above. However, because the adamantyl residue is not spherical, it can fit deeper inside the $\beta$-CD cavity than Figure 2 suggests. The structures suggest that these systems can be useful for measuring the energy difference between the two types of complexes (eq 4).

ITC experiments were carried out for measuring the thermodynamic parameters associated with the inclusion process, $\Delta H^\circ$, $K_{eq}$, $\Delta G^\circ$, and $\Delta S^\circ$. Representative titration experiments are given in Figure 6. It can be noticed that the curves are sigmoidal, the inflection point corresponding to the stoichiometry of the complex formed (1:1 for all guest−host systems). The average values for the thermodynamic parameters are given in Table 1. The experimental relative errors for $\Delta H^\circ$ and $K_{eq}$ (or equivalently for $\Delta G^\circ$) are very low, since they do not exceed 0.7% (with the exception of the rimantidine/3-$NH_2$-$\beta$-CD system for which it is 1%) and 0.5%, respectively. Since $\Delta H^\circ$ and $\Delta G^\circ$ are very close to each other (the maximum difference being 3.5 kJ mol$^{-1}$ (20%) for the rimantidine/3-$NH_2$-$\beta$-CD system), the calculated values for $\Delta S^\circ$ are small and affected by relatively high experimental errors, a rather common fact for these systems.[33]

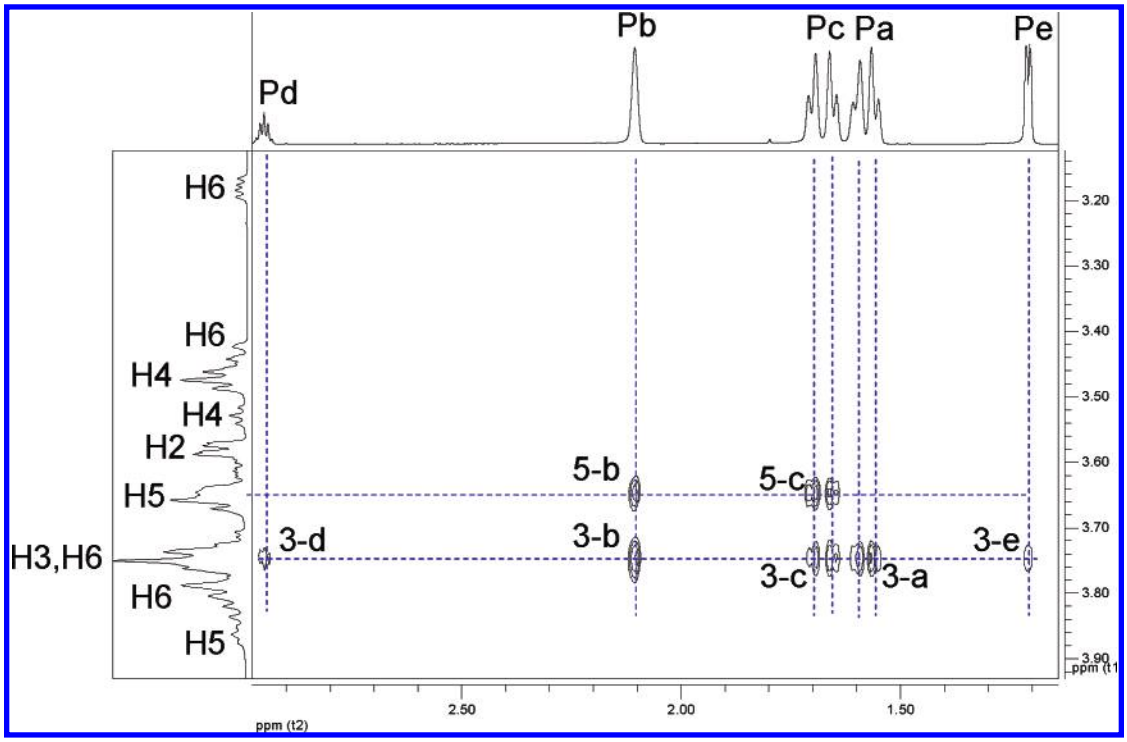



**Figure 5.** Significant cross peaks from the ROE spectrum of the rimantidine/6-$NH_2$-$\beta$-CD system.

**TABLE 3: Thermodynamic Parameters Deduced from Values of Table 1 for the Reactions in Eqs 11 and 12 (see text)**

| | $\Delta G^\circ_-$ (kJ mol$^{-1}$) | $\Delta H^\circ_-$ (kJ mol$^{-1}$) | $T_{303}\Delta S^\circ_-$ (kJ mol$^{-1}$) |
|---|---|---|---|
| reaction in eq 11: g = rimantidine; h″ = 3-$NH_2$-$\beta$-CD; h′ = $\beta$-CD | 11.04 ± 0.11 | 7.73 ± 0.22 | −3.41 ± 0.25 |
| reaction in eq 12: g = rimantidine; h″ = 3-$NH_2$-$\beta$-CD; h = $\beta$-CD | 12.55 ± 0.10 | 11.31 ± 0.10 | −1.36 ± 0.27 |
| reaction in eq 11: g = adamantylmethanol; h″ = 3-$NH_2$-$\beta$-CD; h′ = 6-$NH_2$-$\beta$-CD | 10.85 ± 0.14 | 11.79 ± 0.28 | 1.04 ± 0.31 |
| reaction in eq 12: g = adamantylmethanol; h″ = 3-$NH_2$-$\beta$-CD; h = $\beta$-CD | 11.69 ± 0.11 | 12.80 ± 0.23 | 1.13 ± 0.26 |

For all these systems, the complexation is enthalpy-driven ($\Delta H^\circ < 0$ and $|\Delta H^\circ| \gg |T\Delta S^\circ|$), with $\Delta H^\circ$ values ranging from −17 (rimantidine/3-$NH_2$-$\beta$-CD) to −31 (Ad$CH_2$OH/$\beta$-CD) kJ mol$^{-1}$ and exhibits unfavorable entropy contributions ($\Delta S^\circ <$ 0), with values ranging from −11.7 J mol$^{-1}$ K$^{-1}$ (rimantidine/3-$NH_2$-$\beta$-CD) to −0.5 J mol$^{-1}$ K$^{-1}$ (rimantidine/6-$NH_2$-$\beta$-CD). Our value for $K_{eq}$ for the rimantidine/$\beta$-CD system does not agree with the one published by Vashi and Cukrowski,[9] which seems to be abnormally high when it is compared with other adamantane/cyclodextrin systems.

The differences between the values of the thermodynamic parameters for the complexation of any of the two guests with either $\beta$-CD or 6-$NH_2$-$\beta$-CD are better visualized as the transfer reaction in eq 9 with h = $\beta$-CD, h′ = 6-$NH_2$-$\beta$-CD, and g being any of the two adamantyl derivatives. The values are given in Table 2. It can be noted that the maximum difference in the $\Delta G^\circ_9$ values corresponds to rimantidine with a value of 1.5 ± 0.1 kJ mol$^{-1}$ (6% of the average value of the two free energies from which it is obtained), showing a rather low effect of the charge of the host on the complexation process. This is consistent with the structure proposed for the complex since the positive charges of the guest and host are located on opposite sites of the cyclodextrin cavity and also with the fact that the carbon atom group linked at the 1-position of the adamantyl residue facilitates the hydrophilic group attached to it to protrude toward the bulk solvent. In terms of the equilibrium constant $K_9$, a value of 0.55 is obtained (ideally, it should be equal to 1). The comparison is even better for the neutral guest since $\Delta G^\circ_9 = 0.89 \pm 0.14$ kJ mol$^{-1}$ and $K_9 = 0.70$. It can be concluded that the experimental results suggest that the guests and hosts chosen obey within ±1 kJ mol$^{-1}$, the hypothesis expressed by eqs 8 and 9.

The experimental values for the thermodynamic parameters associated to the formation of a complex by the primary rim of the cyclodextrin (reaction in eq 5) are given in Table 1. In these cases the difference between $\Delta G''^\circ_p$ values for both guests (4.4 kJ mol$^{-1}$) is mainly due to an entropy effect since its unfavorable contribution for the formation of the complex rimantidine/3-$NH_2$-$\beta$-CD is 3.5 kJ mol$^{-1}$ (at the working temperature) while the difference in $\Delta H''^\circ_p$ for both guests is only 0.8 kJ mol$^{-1}$.

Now the remaining parameters outlined in the thermodynamic background can be easily deduced, particularly those related to eqs 11 or 12 (Table 3). The average value of $\Delta G^\circ_-$, obtained from four reactions in eqs 11 and 12, is 11.5 ± 0.8 kJ mol$^{-1}$, which can be considered an excellent estimation for $\Delta G^\circ_-$ (reaction in eq 4). Finally, as a further check of the hypothesis in eq 8, the value of $\Delta G^\circ_{13}$ (reaction in eq 13) can be measured. In this case $c_s \equiv$ adamantylmethanol/$\beta$-CD, $c_s' \equiv$ adamantylmethanol/6-$NH_2$-$\beta$-CD, $c_s^* \equiv$ rimantidine/$\beta$-CD, and $c_s^{*\prime} \equiv$ rimantidine/6-$NH_2$-$\beta$-CD, and the value obtained is $\Delta G^\circ_{13} = 0.67 \pm 0.18$ kJ mol$^{-1}$, which is very close to zero, as expected.

The values for the equilibrium constants for the formation of primary complexes between adamantane derivatives and $\beta$-CD systems estimated here are much higher than many

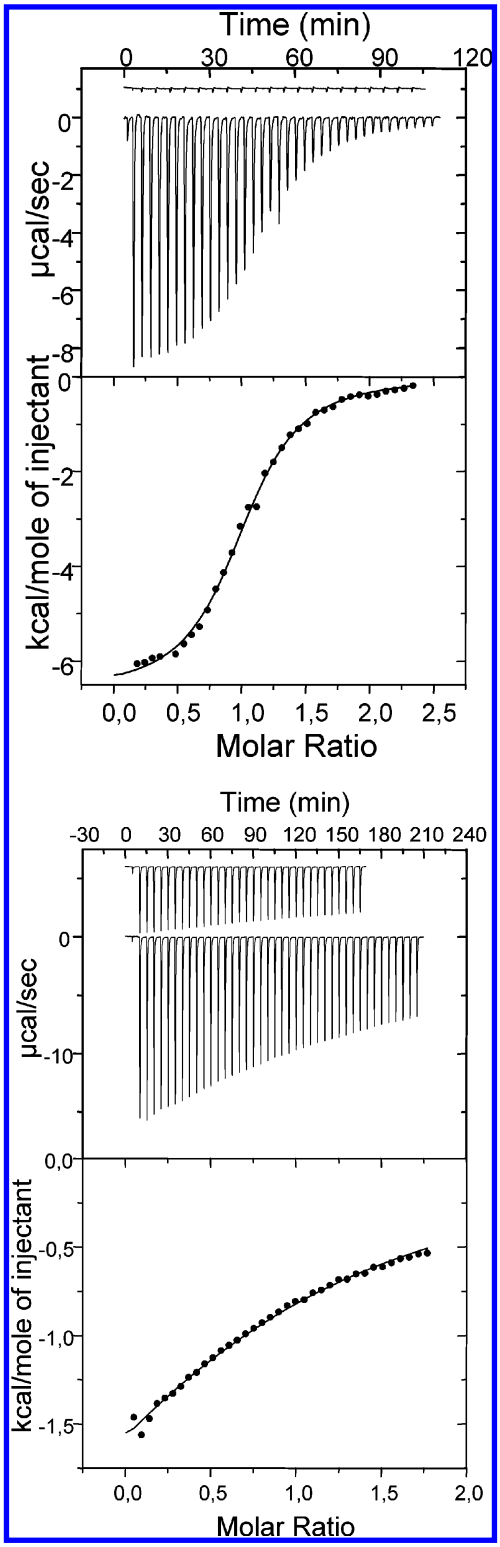



**Figure 6.** Examples of calorimetric titration thermograms: top panel, rimantidine + $\beta$-CD; bottom panel, rimantidine + 3-$NH_2\beta$-CD. Dilution experiments of reactants are shown in the upper plots (above the complexation experiment). Before the results are fit to the theoretical model, dilution contributions are subtracted from the heat effect observed in the corresponding complexation experiment. Note the differences in scales, particularly in the experiment with 3-$NH_2\beta$-CD. The ITC curve for the rimantidine + 6-$NH_2\beta$-CD system (not shown) is identical to the top one. Experimental conditions were as follows: top panel, [$\beta$-CD] = 5 mM (syringe), [rimantidine] = 0.5 mM (cell), pH = 7.0; bottom panel, [3-$NH_2\beta$-CD] = 25.1 mM (syringe), [rimantidine] = 2.85 mM (cell), pH = 5.5. The fitting parameters are given in Table 1.

equilibrium constants for the formation of inclusion complexes of other organic molecules with $\beta$-CD.[33] However, the concentration of the primary complexes, $c_p$, is negligible compared with the concentration of the secondary complexes, $c_s$, representing less than 1% of the total complexes formed. Consequently, its detection by usual experimental techniques is not affordable, and only indirect methods as the one outlined here are useful.

## Conclusion

The H3 and H5 atoms of each glucose residue protrude into the $\beta$-CD cavity forming constrictions to the entrance of guest molecules. In other words, the radii of the heptagons formed by these atoms are shorter than the radii of the primary and secondary entrances to the cavity. This structure suggests that a guest could form two different inclusion complexes corresponding to guest docking to either side of the cavity. However, these two inclusion complexes are not experimentally distinguishable with unmodified $\beta$-CD because one of them is normally preferred. The formation of a given complex can be only guaranteed if the other entrance is blocked. $\beta$-CD was modified by substituting either a primary 6-hydroxyl group or a 3-hydroxyl group with an amino group, obtaining 6-$NH_2\beta$-CD or 3-$NH_2\beta$-CD derivatives, respectively, as guests adamantylmethanol and rimantidine were used. The expected structures of the inclusion complexes formed by the two guests and the three cyclodextrins (native $\beta$-CD, 6-$NH_2\beta$-CD, and 3-$NH_2\beta$-CD) were confirmed by means of ROESY spectra. The free energy of each system was determined from ITC measurements, and from a comparative thermodynamic analysis it was concluded that complexation through the secondary opening is preferred by $11.5 \pm 0.8$ kJ $mol^{-1}$. This explains why only one complex is usually observed in experiments with unmodified $\beta$-CD. The structure of cyclodextrins is frequently characterized as a smooth truncated cone (or a trapezium in the plane), the larger diameter corresponding to the secondary rim. This is an oversimplified picture, which can lead to a misunderstanding of the actual structure of the cavity. The present results suggest that a hyperboloid of revolution can be a better schematic picture to represent the actual geometry of the cyclodextrin cavities.

**Acknowledgment.** The authors thank the Ministerio de Ciencia y Tecnología (MAT2001-2911) and Xunta de Galicia (PGIDIT02PXIC26202PN) for financial support. V.H.S. thanks AECI (Beca Mutis)/University of Costa Rica for a research scholarship.

## References and Notes

(1) Szejtli, J. *Cyclodextrin Technology*; Kluwer: Dordrecht, 1988.
(2) Breslow, R. *Recl. Trav. Chim. Pays−Bas* **1994**, *113*, 493.
(3) Breslow, R. *Pure Appl. Chem.* **1990**, *62*, 1859.
(4) Bender, M. L. In *Cyclodextrins as Enzyme Models in Solution Behavior of Surfactants*; Mittal, K. L., Fendler, E. J., Eds.; Plenum Press: New York, 1982; Vol. 2, p 1171.
(5) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer: Berlin, 1978.
(6) Saenger, W.; Jacob, J.; Gessler, K.; Steiner, T.; Hoffmann, D.; Sanbe, H.; Koizumi, K.; Smith, S. M.; Takaha T. *Chem. Rev.* **1998**, *98*, 1787.
(7) Betzel, C.; Saenger, W.; Hingerty, B. E.; Brown, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7545.
(8) Soto, V. H.; Jover, A.; Galantini, L.; Meijide, F.; Vázquez Tato, J. *Acta Crystallogr., Sect. B* **2004**, *60*, 204.
(9) Vashi, P. R.; Cukrowski, I.; Havel, J. *S. Afr. J. Chem.* **2001**, *54*, 84.
(10) Matsui, Y.; Mochida, K. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 673.
(11) Linnemayr, K.; Rizzi, A.; Allmaier, G. *J. Chromatogr., A* **1997**, *791*, 299.

(12) Harrison, J. C.; Eftink, M. R. *Biopolymers* **1982**, *21*, 1153.
(13) Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1993**, *115*, 9353.
(14) Briggner, L. E.; Ni, X. R.; Tempesti, F.; Wadsoe, I. *Thermochim. Acta* **1986**, *109*, 139.
(15) Eftink, M. R.; Andy, M. L.; Bystrom, K.; Perlmutter, H. D.; Kristol, D. S. *J. Am. Chem. Soc.* **1989**, *111*, 6765.
(16) Gelb, R. I.; Schwartz, L. M. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1989**, *7*, 537.
(17) Godinez, L. A.; Schwartz, L.; Criss, C. M.; Kaifer, A. E. *J. Phys. Chem. B* **1997**, *101*, 3376.
(18) Cromwell, W. C.; Bystrom, K.; Eftink, M. R. *J. Phys. Chem.* **1985**, *89*, 326.
(19) Palepu, R.; Reinsborough, V. C. *Aust. J. Chem.* **1990**, *43*, 2119.
(20) Blandamer, M. J.; Cullis, P. M.; Engberts, J. B. F. N. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2261.
(21) Jannasch, R. *Pharmazie* **1986**, *41*, 478.
(22) Ruediger, V.; Eliseev, A.; Simova, S.; Schneider, H.-J.; Blandamer, M. J.; Cullis, P. M.; Meyer, A. J. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2119.
(23) Gelb, R. I.; Raso, S.; Alper, J. S. *Supramol. Chem.* **1995**, *4*, 279.
(24) Gelb, R. I.; Schwartz, L. M.; Laufer, D. A. *J. Chem. Soc., Perkin Trans. 2* **1984**, 15.
(25) Selvidge, L. A.; Eftink, M. R. *Anal. Biochem.* **1986**, *154*, 400.
(26) Lichtenthaler, F. W.; Immel, S. *Starch* **1996**, *48*, 145.
(27) Rekharsky, M. V.; Goldberg, R. N.; Schwarz, F. P.; Tewari, Y. B.; Ross, P. D.; Yamashoji, Y.; Inoue, Y. *J. Am. Chem. Soc.* **1995**, *117*, 8830.
(28) Cabrer, P. R.; Alvarez-Parrilla, E.; Meijide, F.; Seijas, J. A.; Núñez, E. R.; Vázquez Tato, J. *Langmuir* **1999**, *15*, 5489.
(29) Ladbury, J. E.; Chowdhry, B. Z. *Biocalorimetry. Applications of Calorimetry in the Biological Sciences*; Wiley & Sons: Chichester, 1998.
(30) Kano, K.; Kitae, T.; Shimofuri, Y.; Tanaka, N.; Mineta, Y. *Chemistry* **2000**, *6*, 2705.
(31) Schneider, H. J.; Hacket, F.; Rüdiger, V. *Chem. Rev.* **1998**, *98*, 1755.
(32) Hamilton, J. A.; Sabesan, M. N. *Acta Crystallogr., Sect. B* **1982**, *38*, 3063.
(33) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875.