Experimental (NMR) and Computational (MD) Studies on the Inclusion Complexes of 1-Bromoadamantane with α-, *β*-, and *γ*-Cyclodextrin

P. M. Ivanov,*,† D. Salvatierra, and C. Jaime*

Departament de Química, Facultat de Ciències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Received March 19, 1996[⊗]

The inclusion complexes between the most commonly used cyclodextrins (α-, *β*-, and *γ*-CD) and 1-bromoadamantane were prepared and studied experimentally by NMR methods and by molecular dynamics simulations (AMBER* force field) with solvation. The NMR results suggest host/guest ratios of 2:1, 1:1, and 1:1 for the complexes with α-, *β*-, and *γ*-cyclodextrin, respectively, as well as defined geometries for the complexes. Averaged geometrical data from the molecular dynamics simulations agree with the complexation geometries deduced experimentally.

Introduction

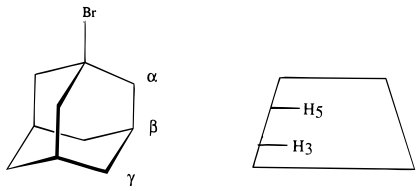
Supramolecular chemistry has attracted the interest of both experimental and theoretical chemists.[1] The binding of molecules without any formal bond between presents a challenge for chemists. The study of such "synthetic" approaches may help us to understand the behavior of the chemical processes in living organisms.

Cyclodextrins, CDs, are cyclic oligomers of α-D-glucose α(1→4) bonded[2] and host molecules with torus-shaped cavities[3] which can form inclusion complexes with neutral organic molecules of different size and functionality.[4] CDs are thus used as enzyme models[5] and in the pharmaceutical industry.[6]

The intrinsic chirality of the CD macrocycles makes them suitable systems for use in studies on chiral recognition. Cyclodextrin derivatives have been largely used as stationary phases for enantiodifferentiation in both gas and liquid−liquid chromatography.[7]

Three main points need to be clarified in the study of inclusion complexes: stoichiometry, association constant, and geometry of the complex. Both the stoichiometry and the association constant can be deduced from experimental data. However, it is not always easy to derive good geometry for the inclusion complexes from the experimental measurements. The inclusion geometry for a given molecule depends on the complexation preference for one specific functional group or molecular fragment (bimodal inclusions)[8−11] and on the conformation of the substrate. Computational methods may give an insight into the energetics of such factors. The combination of experimental and computational studies is a powerful tool for determining of the geometry of complexation.[12−16]

Here we study the inclusion complexes between the three commercially available cyclodextrins (α-, *β*-, and *γ*-CD) and 1-bromoadamantane, **1**. NMR techniques provided experimental evidence of inclusion complexes in solution. The complexation of *β*-CD with **1** was previously studied by NMR and molecular mechanics (MM).[13a] Experimental data for the complexation of **1** with α- and *γ*-CD are presented here, together with results from molecular dynamics (MD) simulations for the three complexes.



1 α-, β-, and γ-CD

Results

In the interpretation of the NMR experiments and MD computations the standard nomenclature for CDs is used, i.e., inner protons are named H^3 and H^5 while outer

† Permanent address: Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, ul. Acad. G. Bonchev, bloc 9, 1040 Sofia.

⊗ Abstract published in *Advance ACS Abstracts,* September 1, 1996.

(1) Vögtle, F. *Supramolecular Chemistry*, John Wiley and Sons, Chichester 1991.

(2) (a) Freudenberg, K.; Meyer-Delius, M. *Ber. Dtsch. Chem. Ges.* **1938**, *71*, 1596. (b) Freunderberg, K.; Plankenhorn, E.; Knauber, H. *Chem. Ind.* **1931**, 731.

(3) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344.

(4) Schlenk, W., Jr. *Fortschr. Chem. Forsch.* **1951**, *2*, 92.

(5) Vögtle, F. *Supramolecular Chemistry: an Introduction*; John Wiley and Sons Ltd.: New York, 1991, p 135.

(6) (a) Szejtli, J. *Cyclodextrins and Their Inclusion Compounds*; Akadémiai Kiadó: Budapest, 1982. (b) Szejtli, J. *Cyclodextrin Technology*, Kluwer Academic Publishers: Dordrecht 1988. (c) Duchêne, D. *New Trends in Cyclodextrins and Derivatives*; Editions Santé: Paris, 1991.

(7) As examples see: (a) Armstrong.; D. W.; Ward, D. J.; Armstrong, R. D.; Beesley, T. E. *Science* **1987**, *232*, 1132. (b) Schürig, V.; Nowotny, H-P. *Angew. Chem., Int. Ed. Engl.* **1990**, 939.

(8) Tee, O. S.; Gadosy, T. A.; Giorgi, J. B. *J. Chem. Soc., Perkin Trans 2* **1993**, *10*, 1705.

(9) Hallen, D.; Schön, A.; Shehatta, I.; Wadsö, I. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 2859.

(10) Isnin, R.; Slam, C.; Kaifer, A. E. *J. Org. Chem.* **1991**, *56*, 35.

(11) (a) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1989**, *111*, 2066. (b) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1989**, *111*, 5138. (c) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1989**, *111*, 7319.

(12) Parrot-Lopez, H.; Galons, H.; Coleman, A. W.; Djedaïni, F.; Keller, N.; Perly, B. *Tetrahedron Asymmetry* **1990**, *1*, 367.

(13) (a) Jaime, C.; Redondo, J.; Sánchez-Ferrando, F.; Virgili, A. *J. Org. Chem.* **1990**, *55*, 4772. (b) Jaime, C.; Redondo, J.; Sánchez-Ferrando, F.; Virgili, A. *J. Mol. Struct.* **1991**, *248*, 317. (c) Fathallah, M.; Fotiadu, F.; Jaime, C. *J. Org. Chem.* **1994**, *59*, 1288. (d) Pérez, F.; Jaime, C.; Sánchez-Ruiz, X. *J. Org. Chem.* **1995**, *60*, 3840. (e) Ivanov, P. M.; Jaime, C. *J. Mol. Struct.* **1996**, *377*, 137.

(14) Myles, A. M. C.; Barlow, D. J.; France, G.; Lawrence, M. J. *Biochim. Biophys. Acta* **1994**, *1199*, 27.

(15) Amato, M. E.; Lombardo, G. M.; Pappalardo, G. C.; Scarlatta, G. *J. Mol. Struct.* **1995**, *350*, 71.

(16) Köhler, J. E. H.; Hohla, M.; Richters, M.; König, W. A. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 319.

S0022-3263(96)00526-9 CCC: $12.00

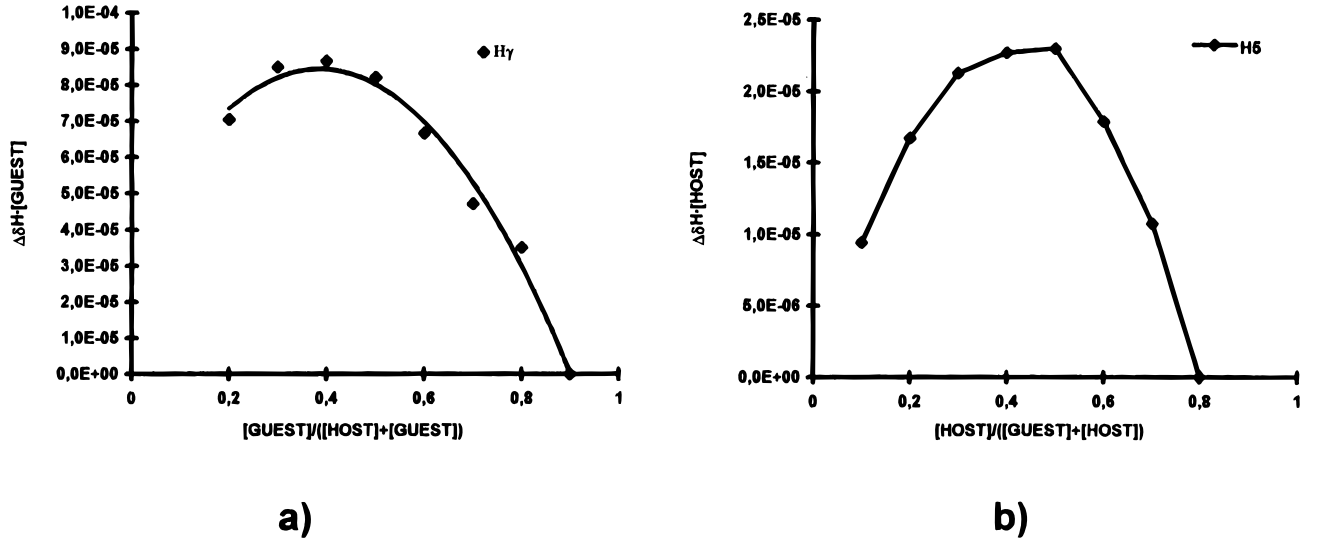


Figure 1. Job's diagram for the α-CD/**1** complex: (a) plot of data corresponding to H^{γ}; (b) plot of data corresponding to H^5.

protons are H^1, H^2, and H^4 (see the simplified structure for α-, *β*-, and *γ*-CD). Protons of **1** are named as H^{α}, H^{β}, and H^{γ} as shown in structure **1**).

NMR Studies. α-Cyclodextrin/1-Bromoadamantane Complex, α-CD/1. Eight samples with host/guest ratios ranging from 0.25 to 9 were prepared as described in the experimental section. ^{1}H-NMR spectra of these samples allowed us to plot Job's diagrams[17] (Figure 1) from which the stoichiometry of the complexes was deduced. Increments in chemical shifts of less than 0.01 ppm were discarded. The Job's diagram for the protons of **1** shows a guest/(host + guest) ratio of about 0.4, indicating more than one host molecule for each guest. The corresponding diagram for the protons of the host shows a host/(host + guest) ratio of 0.5, indicating the presence of one molecule of guest for each host. The average predominant stoichiometry can also be estimated by plotting H^3 or H^5 chemical shift changes against host/guest molar ratios.[18] The most probable host/guest ratio was about 2:1 for any of the protons studied.

Although there are several techniques to predict the geometry of CD inclusion complexes,[19] the ^{1}H−^{1}H nuclear Overhauser effect (NOE)[20] experiment is the most widely used NMR method in spite of the low NOE values obtained. Rotating frame NOE experiments (ROE)[20] give more reliable results. Here 1D- and 2D-ROESY experiments were performed to achieve more detailed information on the molecular geometry of these complexes.

Table 1 lists the ROE enhancements, and Figure 2 depicts the 1D-ROESY. Only values contained in the same line of Table 1 can be used for quantitative comparisons due to inherent ROE conditions. Interestingly, the presaturation of H^3 produced small NOE signals (values between 2.3 and 5.2%) for all of the protons of **1**, while the presaturation of H^5 gave enhancement exclusively on the H^3 cyclodextrin proton. The larger NOE values observed for H^{γ} and H^{α} when compared with those for H^{β} indicate that the latter protons are further from H^3 than H^{γ} and H^{α}. Moreover, on

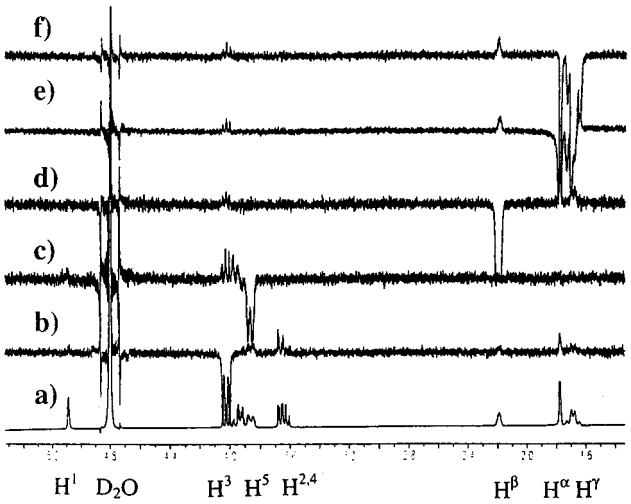


Figure 2. NMR spectra for 1D-ROESY experiments on the α-CD/1 complex: (a) base spectrum; (b) on presaturation of H^3; (c) on presaturation of H^5; (d) on presaturation of H^{α}; (e) on presaturation of H^{β}; (f) on presaturation of H^{γ}.

Table 1. NOE Enhancements (%) Obtained on Presaturation of Different Protons of the α-CD/1 Complex

	observed protons				
presaturated	H^3	H^5	H^{α}	H^{β}	H^{γ}
H^3	−100	5.2	4.5	2.3	5.2
H^5	20	−100	−	−	−
H^{α}	1.66	−	−100	2.8	−
H^{β}	2.85	−	4.6	−100	5.7
H^{γ}	3.2	−	−	4.9	−100

presaturation of the protons of **1**, small but significant values were again observed for H^3 but not for H^5. The average distance between H^{α} and H^3 should be slightly larger than the distance between H^{α} and H^{β} because their relative NOE values are about double (see H^{α} line on Table 1). Intermolecular host/guest distances tended to be greater than the intramolecular distances between pairs of protons of **1**.

These experimental results clearly indicate a position of the guest on the wider rim of the CD cavity, far from H^5 (distances larger than 3.0 Å) and near to H^3 (distances between 2.0−2.5 Å).

(17) Job, P. *Ann. Chim.* **1928**, *9*, 113.
(18) Wood, D. J.; Hruska, F. E.; Saenger, W. *J. Am. Chem. Soc.* **1977**, *99*, 1735.
(19) As a recent example see: Redondo, J.; Frigola, J.; Torrens, A.; Lupón, P. *Magn. Reson. Chem.* **1995**, 104.
(20) Neuhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH: New York, 1989.

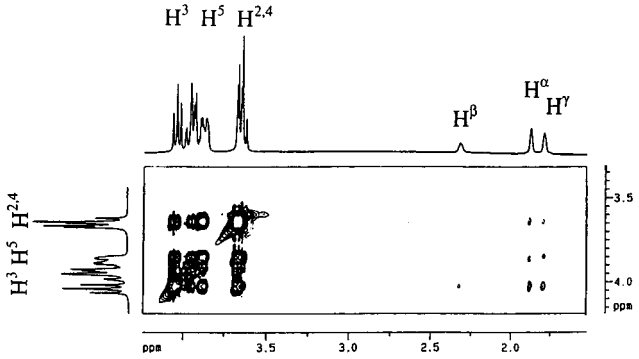


Figure 3. Partial NMR spectrum corresponding to the 2D-ROESY experiments carried out on the α-CD/**1** complex.

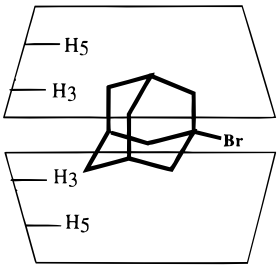


Figure 4. Proposed geometry for the α-CD/1 inclusion complex as deduced from the NMR experimental data based on ROESY experiments and from inspection of molecular models.

The 2D-ROESY experiment (Figure 3) also corroborates this hypothesis. Clear signals are observed for all of the H^3/**1** crossing points, as well as for the H^5/H^α and H^5/H^γ protons.

The absence of signal for the H^5/H^β protons suggests a complexation geometry where the guest is partly included in the host cavity. A possible inclusion geometry can be deduced from inspection of the molecular models: two cyclodextrins encapsulating one guest, with the C−Br bond almost parallel to the glycosidic oxygens plane (Figure 4). In this orientation, all H^β protons are far enough from H^5 (the closest is located on the central axis of the complex) but some of them are near H^3.

Small interactions between the outer CD protons (H^2 and H^4) with the protons of **1** are also observed. They can be a consequence of interactions between nonincluded molecules of **1** and this set of macrocyclic protons.

***β*-Cyclodextrin/1-Bromoadamantane Complex, *β*-CD/1.** The NMR study for this inclusion complex has been already described.[13a] The results from those experiments suggested there may be two types of complex depending on the orientation of the bromine atom respect to the CD cavity, i.e. basal or apical, for which the C−Br bond points toward the wider or narrower CD rim, respectively (see ref 13a).

***γ*-Cyclodextrin/1-Bromoadamantane Complex, *γ*-CD/1.** Eight samples with host/guest ratios ranging from 0.25 to 9 were prepared as indicated in the experimental section. Job's diagrams[17] unequivocally suggest a host/guest ratio of 1:1 (Figure 5).

The 1D- and 2D-ROESY experiments denote the absence of any intermolecular interactions. Thus we were not able to propose a geometry for this complex based on experimental data. Complexes are formed, and this is confirmed by the induced chemical shifts observed on the inner CD protons. We used these chemical shift increments to compute the association constant.[21] The value obtained (64 M^{-1}) indicates a weak intermolecular association.

Computational Studies. The MM minimization studies and the MD simulations were performed using the MacroModel and BatchMin V4.0 software packages.[22] The AMBER* all-atom force field[22,23] and the GB/SA (cavity + van der Waals + electrostatic polarization term) model for water[24] were used in the calculations. Electrostatic interactions were estimated with a dielectric constant 1.0. The computations closely followed calculation protocols tested previously.[13e,25] Extended nonbonded cut-off distances were set to 20.0 Å for the van der Waals and for the electrostatic interactions. Constraints were applied to avoid the guest escapes from the host cavity. Torsional constraints (flat-bottom type, 500 kJ/mol) were imposed (*β*-CD and *γ*-CD) on two dihedral angles that contain C-1 and three alternated glycosidic oxygens. Deviations of 20° and 30° were allowed for the torsional restraints of the *β*-CD and *γ*-CD complexes, respectively. The MD simulations of the complexes of α-CD (exterior complexes[13e]) were carried out by applying distance constraints (*vide infra*) with deviations of 2.0 Å. In addition to the complexes with the guest included, MD simulations were also run for the supramolecules with the guest molecule about 15.0 Å from an average plane of the glycosidic oxygens. The averaged energies from these simulations served as reference points to assess the computed energies of complexation. Energetic restraints to the interatomic distances between C-1 of **1** and three (α-CD) or four (*β*-CD, *γ*-CD) glycosidic oxygens were imposed in these cases with deviations of 1.0 Å. Optimized geometries from an MM study of the complexes[13e] served as starting geometries for the MD simulations. These geometries were obtained from MM modeling of the docking of the guest molecule into the cavity of the macrocycle, assuming different orientations of approach of **1** with respect to the CDs.[13e] The orientations considered were called apical, basal, and parallel depending on the relative orientation of the C−Br bond with the host macrocycle (toward the narrower rim, toward the wider rim, and parallel to the plane formed by the glycosidic oxygens, respectively). Before starting the MD steps, the geometries of the complexes were additionally optimized with the FM-NR minimizer. Starting geometries for 2:1 host/guest complexes of α-CD were generated by adding a second α-CD molecule to computed low-energy 1:1 complexes.[13e] All possible orientations between the two macrocycles were considered: primary−primary, secondary−secondary, and primary−secondary rims of the two macrocycles facing each other with different orientations of **1** embedded between the two macrocycles. The total of twenty 2:1 starting complexes were minimized with the guest between the two macrocycles. More than 20 additional complexes were also studied with starting geometries in which the guest was outside the two CDs, sampling all possible orientations

(21) Salvatierra, D.; Díez, C.; Jaime, C. *J. Incl. Phenom.*, in press.
(22) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
(23) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230.
(24) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.
(25) Ivanov, P. M.; Jaime, C. *Anal. Quim. Int. Ed.* **1996**, *92*, 13.

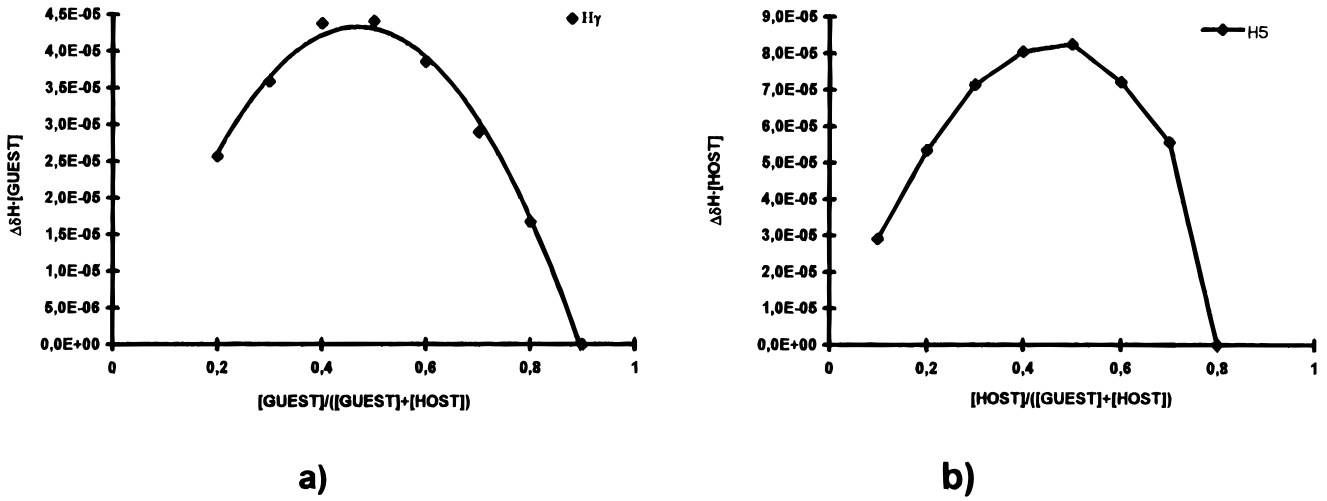


Figure 5. Job's diagram for the γ-CD/1 complex: (a) plot of data corresponding to H^{γ}; (b) plot of data corresponding to H^{5}.

Table 2. Scaled to 300 K Computed Averaged Energies of Complexation (kcal/mol) in Water of 1-Bromoadamantane (1) with α-CD, β-CD, and γ-CD, obtained from MD Simulations using the AMBER* Force Field

	orientation of approach of **1**	α-CD	β-CD	γ-CD
total energy	apical	−4.5	−13.1	−6.3
	basal	−7.0	−14.6	−14.9
	parallel	−8.5	−12.1	−14.3
van der Waals	apical	−10.6	−13.9	−11.2
	basal	−10.3	−12.5	−11.7
	parallel	−7.6	−13.9	−11.6
electrostatic	apical	−0.3	2.0	3.2
	basal	−1.1	−1.1	−0.1
	parallel	−0.3	2.6	−0.1
solvation	apical	3.0	2.8	2.2
	basal	3.4	3.1	3.8
	parallel	1.5	2.7	3.9

between the two CDs and **1**. Due to limitations in the computational resources, these were studied only with MM.

The initial velocities assigned for the MD runs correspond to 5.0 K. Three consecutive MDYN commands[25] were executed in all cases. The first step involves 20.0 ps integration with a 0.3 fs time step at the constant temperature of 300 K, and it is a preliminary equilibration run. A second equilibration step at the same temperature was also included. For this MD run, 0.3 fs time step and 100.0 ps for equilibration were provided, thus allowing more even distribution of the energy throughout the large number of degrees of freedom. The third MD step is the actual MD simulation of 1.2 ns with a 0.5 fs time step at 300 K. A total of 200 structures were saved during this run and later examined with the XCluster program.[26] The averaged steric energies scaled to the bath temperature 300 K are the quantities we used to compare the relative stabilities of different complexes. All MD steps used SHAKE operating on all bonds. In order to eliminate accumulated errors from integration during the 1.2 ns simulations due to contributions from bond stretchings, we ran an additional 200.0 ps simulation with sampling every 1.0 ps. The results from the MD simulations are presented in Table 2. These are computed averaged energies of complexation in water, as well as the contributions from the most important energy terms (van der Waals, electrostatic, and solvation energy contributions). Stereoscopic views are presented in Figure 6 of the lowest energy 2:1 host/guest complexes of α-CD with **1** obtained after full geometry optimization without imposing any restrictions. In Figure 7 are given clustered molecular displays of the dynamics of the complexes of α-CD, β-CD, and γ-CD with **1**. The clusterings include only the last 50 structures of the samplings that refer to the time interval of simulation from the 900th to the 1200th ps.

The energies of complexation estimated from the MD simulations (Table 2) corroborate the conclusions from the previous MM minimization studies of the 1:1 complexes.[13e] The energy of complexation of β-CD and γ-CD with **1** are practically the same and almost double the energy stabilization upon complexation with the smaller α-CD. MD simulations gave average energies about 4 kcal/mol greater than MM studies[13e] in all cases. The MD simulations confirm the conclusion[13e] for the prevailing contribution of the van der Waals interactions for the formation of the 1:1 complexes. In all cases considered and within the frames of the solvation model used, the solvent favors the separated host and guest. The electrostatic interactions also make a significant contribution to the balance of interactions between the guest and the host macrocycle, and they can favor or disfavor the complexation depending on the orientation of approach of the guest molecule. Thus, it is very important that all possible orientations of approach of the guest should be examined in such studies.

In accord with experimental evidence, the MD simulations for β-CD and γ-CD show that the guest is inside the torus (Figure 7b,c; inner 1:1 complexes of β-CD and γ-CD with **1**). In the case of the smallest macrocycle, α-CD, the guest is outside the torus (exterior 1:1 complex of α-CD with **1**). Distance monitoring for different pairs of protons on **1** and the CDs was also performed during the MD simulations. The shortest average distances obtained for γ-CD are about 2.8 Å, which is in line with the 1D-and 2D-ROESY experiments for the γ-CD/1 complex (*vide supra*), in which intermolecular interactions were not observed. Thus, the torus of γ-CD is too large for short contacts between protons of the guest molecule and the macrocycle to manifest as NOE enhancements in the spectra. An averaged distance of 2.5

(26) Shenkin, P. S.; McDonald, D. Q. *J. Comput. Chem.* **1994**, *15*, 899.

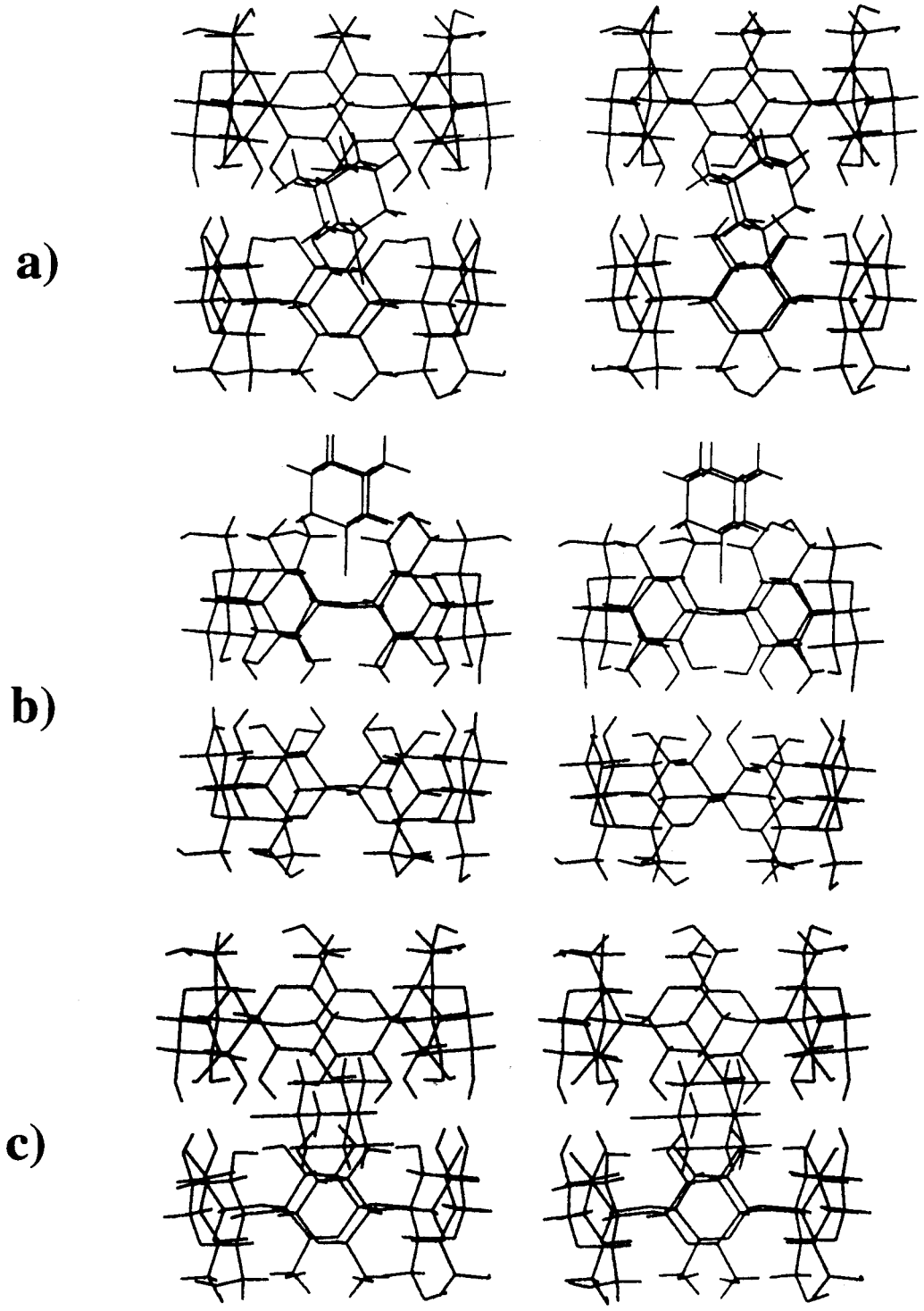


Figure 6. Stereographic views of the lowest energy 2:1 host/guest complexes of α-CD with **1** obtained by MM calculations after full geometry optimization without imposing any restrictions: (a) most stable complex; (b) outside complex; (c) complex with the C−Br bond parallel to the macrocycle planes.

Å was obtained for an H^5/H^β pair, and between an H^α and a methylene proton of a CH_2OH group, for a complex of α-CD. If short contacts are indeed present in the 1:1 α-CD/**1** complex, it will be difficult to separate that effect from the stronger enhancements resulting from the 2:1 complexes.

For the *β*-CD/1 complex, where the guest molecule is totally embedded into the macrocycle, and the diameter of the macrocyclic ring is not as large as *γ*-CD; several short average proton/proton distances were computed ranging from 2.40 Å to 2.55 Å for pairs of the H^5/H^α, H^5/H^β, and H^5/H^γ type. For other orientations of approach, the H^3 protons are always nearer than H^5 to their respective pair. The larger flexibility of the *γ*-CD macrocycle[13e] is also manifested by the MD simulations: the largest values of average dihedral angles between glycosidic oxygens are about 40° for *γ*-CD, while for α-CD and *β*-CD these are ca. 2° and 11°, respectively.

The results for the 2:1 complexes of α-CD with **1** obtained by MM minimization are very interesting. They facilitated the interpretation of the NOE data. The lowest energy complex was computed to have the guest

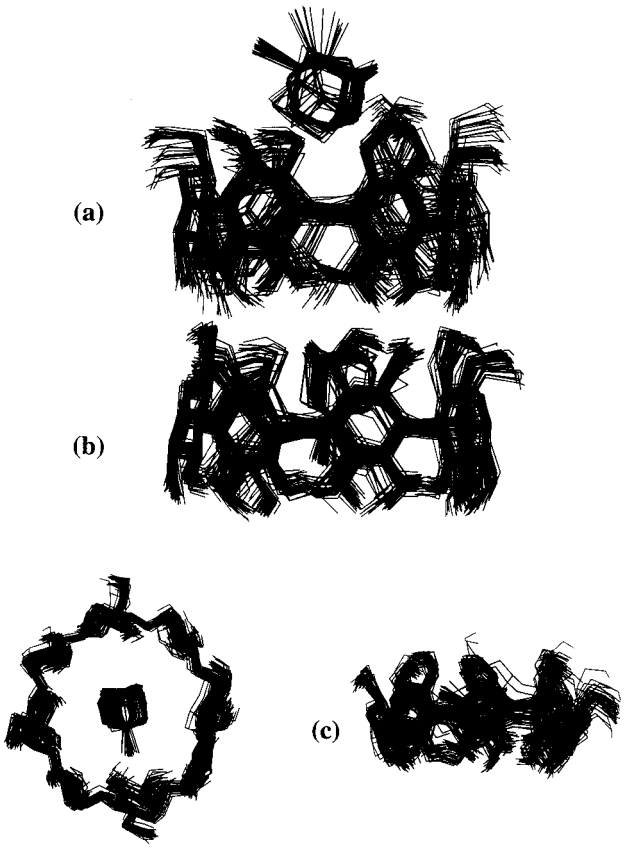


Figure 7. Clustered molecular display. Dynamics of the 1:1 complexes of α-CD (a**),** *β*-CD (b), and *γ*-CD (c) with 1-bromoadamantane. The drawings include only 50 structures that refer to the time interval of simulation from the 900th to the 1200th ps. The starting geometries for the simulations were obtained from MM minimizations following parallel (α-CD) and basal (*β*-CD and *γ*-CD) approaches of the guest molecule.[13e]

molecule between the two CDs, oriented almost perpendicular to the average planes of the macrocycles (Figure 6a). Another complex, which has the guest outside the CD-dimer (Figure 6b), has practicaly the same energy. The former complex is stabilized almost exclusively by the van der Waals interactions (ca. 6.5 kcal/mol), while the latter has more favorable solvation (1.7 kcal/mol) and angle bending (3.3 kcal/mol) contributions. The structure with the C−Br bond parallel to the planes of the macrocyles, and the guest flanked by the two CDs (Figure 6c), has about 6.5 kcal/mol more than the other two. The most significant destabilizing energy contribution for this complex resulted from the electrostatic interactions. Thus, quite a delicate balance between different energetic contributions governs the stabilities of the complexes. Short distances of 2.1−2.2 Å were obtained for H^{γ}/H^{5} pairs in the last case, while such NOE enhancements are lacking in the spectra (Table 1). For the perpendicular orientation (Figure 6a) however, all computed short distances are in agreement with the experimental findings. The H^{3}/H^{α} and H^{3}/H^{γ} distances are about 2.1−2.2 Å and 2.25 Å, respectively, while the contacts of H^{β} with H^{3} protons range from 2.32 Å to 2.60 Å. Indeed, one of the NOE enhancements (Table 1, H^{β}) is weaker than the other two.

Conclusions

The inclusion complexes between 1-bromoadamantane, **1**, and α- and *γ*-CD have been prepared. Their NMR study allowed us to deduce a 2:1 and 1:1 stoichiometry, respectively, with an association constant of 64 mol^{-1} for the *γ*-CD/1 complex. Molecular modeling techniques (MM and MD calculations) combined with experimental NMR data are powerful tools for the study of CD inclusion complexes. They provide valuable geometrical and energetic data for the determination of the final geometry of inclusion.

Experimental Section

NMR spectra were recorded in a 400 MHz spectrometer at the "Servei de Ressonància Magnètica Nuclear de la Universitat Autònoma de Barcelona". ROESY experiments were recorded using the 1:1 (or 2:1 for α-CD/**1**) samples. The experiments were carried out at 750 ms mixing time.

Cyclodextrins were available commercially from Jansen and were used without further purification. 1-Bromoadamantane was obtained from Aldrich.

General Procedure. A solution of **1** in D_2O was prepared by dissolving 10 mg of **1** in 10 mL of D_2O under sonication. Solutions of **2** and **4** in D_2O having a concentration of 9.6×10^{-2} M were also prepared. Fixed volumes of the solutions of **1** and **2** (or **1** and **4**) were mixed to give a predetermined final volume (usually 1 mL). The relative host/guest proportion were confirmed from direct integration of their NMR signals.

Acknowledgment. The "Servei the Ressonància Magnètica Nuclear de la Universitat Autònoma de Barcelona" is acknowledged for the facilities in allocating spectrometer time. Financial support was obtained from DGICYT (Ministry of Education and Science, Spain) through grant no. PB92-0611. CIRIT (Generalitat de Catalunya, Catalonia, Spain) is also acknowledged for a Visiting Professor grant to one of us (P.M.I.).

JO960526V