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# Investigation of the Complexation between 4-Aminoazobenzene and Cucurbit[7]uril through a Combined Spectroscopic, Nuclear Magnetic Resonance, and Molecular Simulation Studies

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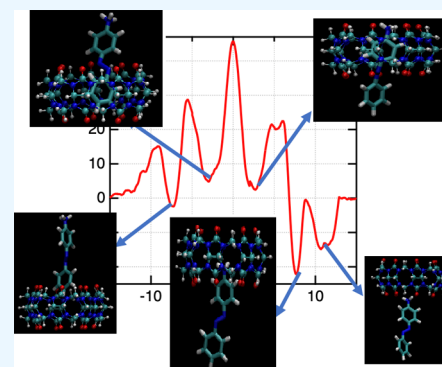


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Supporting Information

**ABSTRACT:** Cucurbiturils are well known for their ability to form supramolecular systems with ultrahigh affinities binding. Inclusion complex between 4-aminoazobenzene and cucurbit[7]uril has been investigated in aqueous solution by ultraviolet (UV)-spectroscopy,  $^1\text{H}$  NMR, and molecular simulations. 4-aminoazobenzene shows high affinity in acidic solutions while no association was detected in neutral solutions. The thermodynamic properties of complex formation are investigated using both UV spectroscopy and nuclear magnetic resonance (NMR) measurements. Our results highlight that the high binding constant between CB7 and 4AA ( $\log K = 4.9$ ) is the result of a large negative change in  $\Delta_r H^\circ$  ( $-19$  kJ/mol) and a small positive change in  $T\Delta_r S^\circ$  (9 kJ/mol). The analysis of the experimental data lead to hypothesis on the structure of the complex. We have used molecular dynamics simulation to interpret experiments. Interestingly, the *cis*–*trans* isomerization of aminoazobenzene is considered. All the results are discussed and compared with those previously obtained with other host molecules.



## 1. INTRODUCTION

Supramolecular recognition results from an association between two molecules (host and guest), leading to a molecular complex.<sup>1,2</sup> This association is controlled by reversible processes involving many weak force interactions (electrostatic, cation– $\pi$ , hydrogen bonds, van der Waals, and hydrophobic effects) and is characterized by its binding constants and also related thermodynamic quantities. The most extensively used host molecules are cyclodextrins,<sup>3,4</sup> calixarenes,<sup>5–7</sup> and cucurbiturils.<sup>8–10</sup> Most of the diverse life sciences and pharmaceutical applications of this particular chemistry are in aqueous media.<sup>11,12</sup> Therefore, the receptor molecules or hosts must have good solubility in water. For this purpose, a substituent can be added to the macrocyclic hosts to enhance the solubility. This is the case of *p*-sulfonatocalix[*n*]-arene.<sup>13,14</sup> Interestingly, the inclusion capabilities of these water-soluble calixarene derivatives are significantly higher as those of unmodified calixarenes.<sup>15</sup> With regard to the cucurbituril family (CB), their solubility is generally lower than that of cyclodextrins.<sup>16</sup> However, cucurbit[5]uril (CB5) and cucurbit[7]uril (CB7) reach solubility in pure water of 20–30 mM. In contrast, the water solubility of CB6 and CB8 is 0.018 and <0.01 mM, respectively.<sup>17</sup> In addition to its good solubility in water, CB7 are commonly known to show strong binding affinity with certain guest molecules,<sup>17</sup> making it an important member of the cucurbituril family.

Although these three classical water-soluble macrocycles have a hydrophobic internal cavity, properties such as shape and flexibility differ significantly, the cavity of the *p*-sulfonatocalixarenes being the most flexible of the three molecules.<sup>18,19</sup> Regarding the sizes of the cavities, that of CB6, CB7, and CB8 are identical to those of  $\alpha$ -cyclodextrins ( $\alpha$ -CD),  $\beta$ -cyclodextrins ( $\beta$ -CD), and  $\gamma$ -cyclodextrins ( $\gamma$ -CD), respectively. However that of CB is equatorially symmetrical, and consequently, both cavity openings are identical. In contrast, the other two macrocycles (CDs and calixarenes) have cavity openings that differ both in size and chemical nature. Not surprisingly, the driving forces for guest inclusion in the cavity depend strongly on the nature of the host molecule. Thus, the hydrophobic effects are mainly responsible for the encapsulation of the guest inside the inner cavities of CDs. Although the hydroxyl groups of the cyclodextrin portal are able to form hydrogen bonds, especially with anionic guest molecules, they do not really have strong interactions with most included guests. For *p*-sulfonatocalixarenes, the driving

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forces for the binding process are both electrostatic and  $\pi$ -stacking interactions with cations. The high-affinity binding of the included guest cations is known to be the result of some effects for CBs. These effects are the size complementary between the host and guest, the hydrophobic effect,<sup>17</sup> and also the ion-dipole interactions with positively charged cationic guests (in particular, ammonium groups) because of the presence of the electronegative carbonyl portals of CBs.<sup>20</sup>

Many authors have been interested in CBs because of their robust abilities to bind guests in their cavities in aqueous solution (neutral compounds,<sup>18</sup> dyes,<sup>19</sup> ferrocene derivatives,<sup>20</sup> or drugs<sup>21</sup>), which is not the case with more conventional hosts molecules (calixarenes and cyclodextrins). For azoalkanes, Guo et al.<sup>18</sup> have shown that CBs form stable complexes; their association constants being 2–5 orders of magnitude higher than  $\beta$ -CD. The comparative study between the complexes of ferrocene derivatives with CB7 and  $\beta$ -CD carried out by Jeon et al.<sup>20</sup> have shown that the CB7 complex is more stable with positively charged ferrocene than that formed with  $\beta$ -cyclodextrin. Indeed, the association constant of CB7 is in the  $10^9$  to  $10^{13}$  range (while binding constants of cyclodextrin inclusion complexes with ferrocene derivatives are in the  $10^3$  to  $10^4$  range). It should be noted that no association was observed between CB7 and negatively charged ferrocene derivatives.

Among the guest molecules studied, the azobenzene derivatives have often been studied for their ability to obtain driven molecular machines.<sup>22–26</sup> However, its poor solubility in water poses problems in expanding its applications in biology. To circumvent this problem, the introduction of the amino group yielded a slight increase in water solubility.<sup>27</sup> However, the solubility of 4-aminoazobenzene (4AA) in pure water remains around 0.15 mmol/L at 25 °C.<sup>28</sup> To further increase its solubility, it would be possible to use a mixture of water with another organic solvent. However, the amount of cosolvent must be as low as possible to reduce the change in the association properties.<sup>29–32</sup> Surprisingly, there are a few studies on the evaluation of the supramolecular interaction of azo compounds with cucurbiturils.<sup>33–35</sup> To the best of our knowledge, there are only one report on the CB7 about the host–guest complexation of cucurbit[7]uril (CB7) and 4,4'-diaminoazobenzene as a function of pH.<sup>35</sup>

In a preliminary work, we had investigated the association between 4AA and three different host molecules ( $\beta$ -CD, *p*-sulfonatocalix[*n*]arene with *n* = 4,6) in water.<sup>27</sup> We had applied UV–visible spectroscopy to obtain the fully thermodynamical characterizations ( $K$ ,  $\Delta_r G^\circ$ ,  $\Delta_r H^\circ$ , and  $T\Delta_r S^\circ$ ) of the association between hosts and 4AA. The complexation behaviors were always enthalpically favored with a rather weak association for all complexes studied. Indeed, the association was taken place regardless of the form of 4-aminoazobenzene (protonated or not) with  $\beta$ -cyclodextrin while only the complex in acidic solutions (protonated guest) was observed with *p*-sulfonatocalix[*n*]arene.<sup>27</sup> We aim here to study the association between CB7 and 4-aminoazobenzene (4AA) in water. Our goal is to obtain a significantly larger binding constant than that previously obtained with other hosts. Because careful choice of the stoichiometric binding model for investigating the structure and thermodynamic properties of the complex is essential, we have first used the job plot method<sup>36</sup> to unambiguously elucidate the stoichiometry of the studied system. Second, all the thermodynamic parameters ( $K$ ,  $\Delta_r G^\circ$ ,  $\Delta_r H^\circ$  and  $T\Delta_r S^\circ$ ) of the association complex have

been obtained by both UV–visible spectroscopy and NMR measurements. Finally, a molecular simulation approach is implemented to obtain both structural and energetical insights into the complex. All these results are discussed with previous data obtained with other host molecules<sup>27</sup> or other guest molecules.<sup>35</sup>

## 2. EXPERIMENTAL SECTION

**2.1. Materials.** Cucurbit[7]uril (CB7), HCl, and DCl were provided by Sigma Aldrich. Water quantities of CB7 were determined with Karl Fisher (Mettler-Toledo, DL32). For 4-aminoazobenzene (4AA) and D<sub>2</sub>O, their suppliers were, respectively, TCI and Eurisotop. All the compounds have a purity of at least 97% and were used without any purification.

Two solutions of concentrations 0.02 and 0.2 mmol/L of 4-aminoazobenzene were prepared at two different pH (1 and 7) using deuterated solvents for nuclear magnetic resonance (NMR) solutions. To facilitate the solubilization of the guest molecule, we have added a small amount of acetonitrile 0.04 and 0.3% by volume. Host solutions from 0.03 to 0.72 mmol/L were prepared either in acidic solutions using concentrated HCl (or DCl and CND<sub>3</sub> for NMR) or neutral solutions using distilled water (or D<sub>2</sub>O and CND<sub>3</sub> for NMR). pH measurements (MP120, Mettler-Toledo) were made for all prepared solutions.

**2.2. Ultraviolet Spectroscopy Measurements.** An ultraviolet (UV)–visible (vis) spectrophotometer (Jasco V650) equipped with Peltier thermostatisation (ETCS-761) was used to follow the change in absorption intensity while increasing CB7 concentrations in aqueous solutions. The measurements were performed at four temperatures (288, 298, 308, and 323 K). The association between CB7 and 4AA was calculated using Benesi–Hildebrand's equation<sup>37</sup> (1):

$$\frac{[4AA]_0[CB7]_0}{\Delta E} = \frac{1}{K\Delta\epsilon} + \frac{[CB7]_0}{\Delta\epsilon} \quad (1)$$

Then, the free Gibbs energy change for inclusion complexes is given by the following equation:

$$\Delta_r G^0 = -RT \ln K \quad (2)$$

Finally, the thermodynamic parameters were calculated from van't Hoff eq 3 as follows:

$$\frac{d\ln K}{dT} = \frac{\Delta_r H^0}{RT^2} \quad (3)$$

**2.3. NMR Spectroscopy Measurements.** The <sup>1</sup>H NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer equipped with Bruker 5 mm inverse probe TXI (<sup>1</sup>H/<sup>13</sup>C/<sup>15</sup>N) with a z-gradient coil probe. Solution of tetra-deuterated trimethylsilylpropionate (TSPd<sub>4</sub>) in D<sub>2</sub>O was used as an internal reference for chemical shifts. To eliminate possible interactions between TSPd<sub>4</sub> and the host molecule, the internal reference was introduced into a coaxial insert itself placed in the NMR tube. For all samples, a one-dimensional <sup>1</sup>H NMR spectrum was acquired using a NOE spectroscopy sequence (NOEsygppr1d with water presaturation and gradients) with low power irradiation of water resonance during the recycle delay of 4 s and the mixing time of 10 ms. Scans (256) were collected with an 90° impulsion time of 11.5 μs, an acquisition of 3.28 s, a spectral window of 10,000 Hz, and 64 K data points zero-filled to 128 K before Fourier transformation with 0.3 Hz line broadening. For 4AA, two-

dimensional (2D)  $^1\text{H}$ - $^1\text{H}$  COSY homonuclear experiment (cosygpqrqf sequence with water presaturated and gradients) was performed at 298 K with quadrature phase detection in dimensions using the QF detection mode in the indirect one. Increments (384) in the indirect dimension were obtained, 4 K data points were collected, and 64 transients were accumulated in the direct dimension. A square sine-bell function was applied in the two dimensions before Fourier transformation. By following the change in the chemical shift under different concentrations of host molecules, we calculate the association constant using the linearization approach.<sup>38–40</sup> The measurements were made at four temperatures: 288, 298, 308, and 323 K.

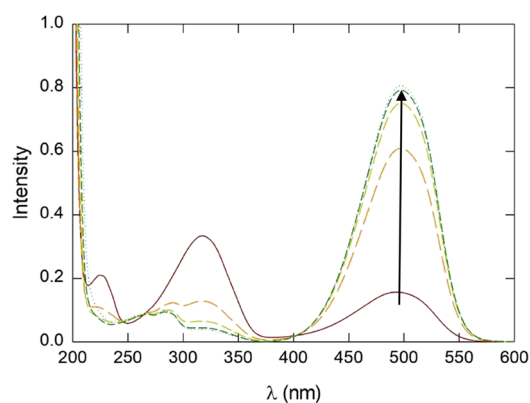
**2.4. Molecular Simulation.** All the MD simulations presented in this study have been performed in acidic solutions. Quantum calculations have been used to calculate the partial charges of the CB7 molecule with the M062X method with the 6-31G(d,p) base. A cubic simulation box of 20 Å length was used and composed with two thousand water molecules, one 4AA, and one CB7 molecule. pH is taken into account using protonated 4AA (protonation takes place in the azo group).  $\text{Cl}^-$  was added in order to maintain the neutrality of the solution. AMBER force field<sup>41</sup> was chosen for guest and host molecules, and the SPCE dehydration model was used for water. The pressure and temperature were fixed, respectively, to 1 atm and 300 K. A cut off radius of 8 Å was applied for short-range interactions. The MD simulations were performed with the LAMMPS package<sup>42</sup> first with an equilibration step in the NVT ensemble of 1 ns followed by an acquisition time in NPT of 20 ns.

PMF calculations has been carried out with the umbrella sampling (US) method<sup>43–45</sup> with both isomers. Initially, these calculations were carried out with the most stable *trans* isomer, then with the *cis* form by blocking the C–N–N–C dihedral. The simulation box length has been increased up to 57 Å with more than 6000 water molecules. The cut off has been also increased to 10 Å. PMF simulations has been performed using the LAMMPS package with the COLVARS module. The PMF has been defined as the distance between the centers-of-mass of CB7 and 4AA. The transversal freedom of move has been limited in order to get a valid PMF result. A global equilibration in the NPT ensemble was performed for 100 ps and then a NVT one for 100 ps. The PMF was run in the NVT ensemble and was separated into six different stages. Each stage was composed of an equilibration of 200 ps and an acquisition of 10 ns. The 6 stages sample the distance between –20Å and 20Å with overlap to help the reconstitution of the PMF curve.

### 3. RESULTS AND DISCUSSION

UV spectroscopy measurements are performed as a function of pH. First, we have examined the interaction between 4AA and CB7 at neutral pH (Figure S1). As already observed with *p*-sulfonatocalixarene,<sup>27</sup> no association is detected in the neutral medium.

Second, this association in acidic solutions at pH 1 is examined (Figure 1). At pH 1, protonation of 4AA takes place in the amine group ( $\text{p}K_a$  value of 2.82 at 25 °C<sup>46</sup>). Additionally, it seems that CB can also help to the protonation of guest.<sup>35,47</sup> Indeed, Lazar et al.<sup>47</sup> shows that CBs can shift the  $\text{p}K_a$  value of the guest upon inclusion, leading to better binding. The adsorption spectra of 4AA at pH 1 (Figure 1) clearly differ from that in neutral solution (Figure S1), showing

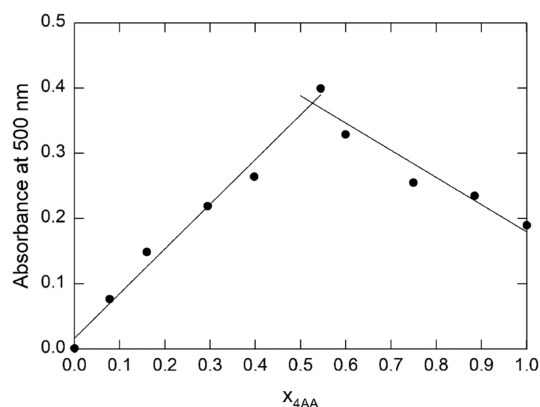


**Figure 1.** UV absorption spectra of 4AA (0.02 mM) in acidic solutions with increasing concentrations of CB7 (0.03–0.3 mM).

peaks at different wavelengths. This spectra in acid pH solutions is in accordance with that obtained by Ventakesh et al.<sup>48</sup> They demonstrate that there exists a tautomeric equilibrium between the ammonium and azonium form for the protonated 4AA, the band at 500 nm being assigned to the azonium cation.

It is worth noting that a large increase in absorbance at 500 nm is observed with the increase in the CB7 concentration (Figure 1).

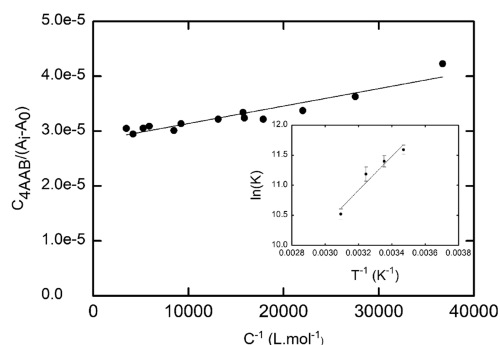
In order to determine the stoichiometry of the studied system, we use the job plot method.<sup>36</sup> For this, the total concentration is kept constant ( $C_{\text{CB7}} + C_{\text{4AA}} = 0.02 \text{ mmol L}^{-1}$ ) and varying the host and guest concentrations. Figure 2 represents the absorbance of the system at 500 nm as a function of the 4AA mole fraction. The results show a maximum around 0.5, confirming a 1:1 stoichiometry.



**Figure 2.** Job plot method: absorbance at 500 nm as a function of the guest mole fraction.

Knowing the formation of 1:1 complex in acidic solutions, the log  $K$  values are determined as a function of temperature using the Benesi–Hildebrand<sup>37</sup> eq 1. Then, the enthalpy of the system is calculated with van't-Hoff relation 3 (Figure 3). All these thermodynamic properties are reported in Table 1.

To confirm these results and to obtain further insight into the complex structure,  $^1\text{H}$  NMR experiments in  $\text{D}_2\text{O}$  are also performed. The 4AA spectrum with various amounts of CB7 obtained in neutral medium are reported in Figures S2 and S3 with different concentrations of 4AA. Let us note that the NMR spectra of the pure guest (Figure S4) and those of the



**Figure 3.** Benesi–Hildebrand plot at 25 °C Inset: Van't Hoff Plot  $R^2 = 0.94$ .

**Table 1.** Thermodynamic Parameters Characterizing the Association of 4-Aminoazobenzene by the  $\beta$ -Cyclodextrin, Calixarenesulfonates, and Cucurbit[7]Uril in both Neutral and Acidic Solutions at 298 K

hosts	log $K$ (25 °C)	$\Delta_r G^\circ$ kJ mol <sup>-1</sup>	$\Delta_r H^\circ$ kJ mol <sup>-1</sup>	$T\Delta_r S^\circ$ kJ mol <sup>-1</sup>
pH = 7.2				
$\beta$ -Cd <sup>27</sup>	3.35 ± 0.05	-19 ± 1	-8 ± 1	11 ± 2
$\beta$ -Cd <sup>49</sup>	3.33	-19		
C4S <sup>27</sup>	no association detected			
C6S <sup>27</sup>	no association detected			
CB7	no association detected			
pH = 1				
$\beta$ -Cd <sup>27</sup>	2.7 ± 0.5	-15 ± 1	-5 ± 2	11 ± 3
C4S <sup>27</sup>	2.0 ± 0.5	-11 ± 3	-25 ± 2	-13 ± 5
C6S <sup>27</sup>	2.5 ± 0.5	-14 ± 3	-18 ± 2	-4 ± 5
CB7 <sup>a</sup>	4.9 ± 0.2	-28 ± 2	-19 ± 7	9 ± 8
CB7 <sup>b</sup>	4.4 ± 0.1	-26 ± 2	-24 ± 4	1 ± 4

<sup>a</sup>Obtained from UV spectroscopy. <sup>b</sup>Obtained from NMR measurements.

pure host (Figure S5) are in perfect agreement with those of the literature in D<sub>2</sub>O solution.<sup>21,40</sup> In the CB7 spectrum, two doublets with chemical shifts around 5.8 and 4.3 ppm are obtained. These doublets correspond to methylene groups that are located on the portals of the molecule. Additionally, the CH group situated on the equatorial plan of symmetry of CB7

appears as a singlet around 5.6 ppm. Clearly, the evolution of the <sup>1</sup>H NMR spectra of 4AA at pD 7 in the presence of an increasing amount of CB7 did not show any change in line with the results found by UV spectroscopy and our previous experimental and simulation studies with *p*-sulfonatocalix[*n*]-arene<sup>27</sup> or with other guest molecules.<sup>21</sup>

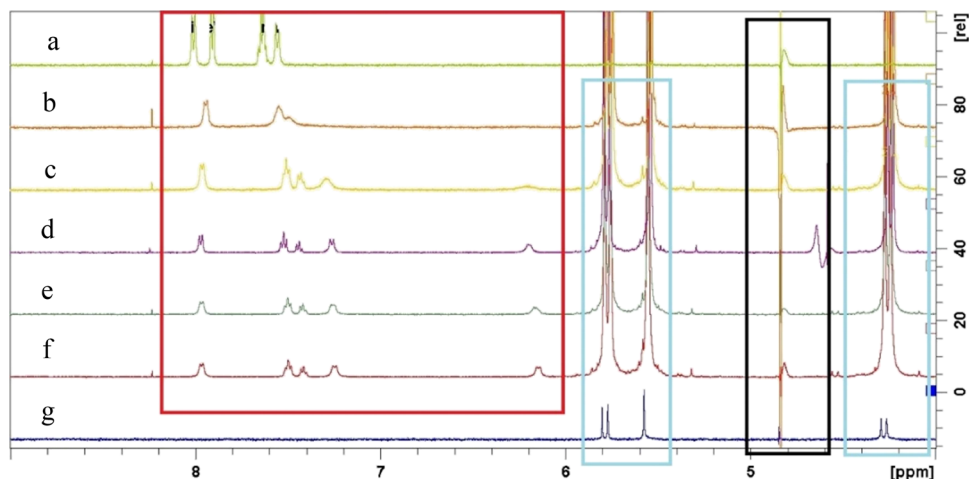
In a second step, we have studied the evolution of the <sup>1</sup>H NMR spectra in acidic solutions at pD 1 and 298 K (Figure S6). Appreciable changes in the chemical shift values of the guest protons are often observed in these systems if the guest molecules are included in the host cavities.<sup>21,40,49,50</sup> As shown in Figure S6, we observe both a modification of the proton peaks of the guest molecule and a slight displacement of those of the host molecule as increasing amounts of CB7 are added.

Similar analysis is performed with a more concentrated 4-aminoazobenzene solution to obtain larger chemical shifts (Figure 4). A large modification of the peaks associated to the guest protons is observed while increasing the CB7 concentration. This is consistent with the formation of a complex as observed by UV spectroscopy. Moreover, this modification highlights a change in the proton environment of the guest molecule, as observed by Gamal-Eldin et al.<sup>50</sup> on the host–guest complexations of amine boranes and isoelectronic/isostructural quaternary alkylammonium cations by CB7 in aqueous solution. Their results show that an upfield shift indicates that the guest proton is located within the internal cavity, while a downfield shift shows that the proton is rather near the carbonyl groups of a portal.<sup>50</sup>

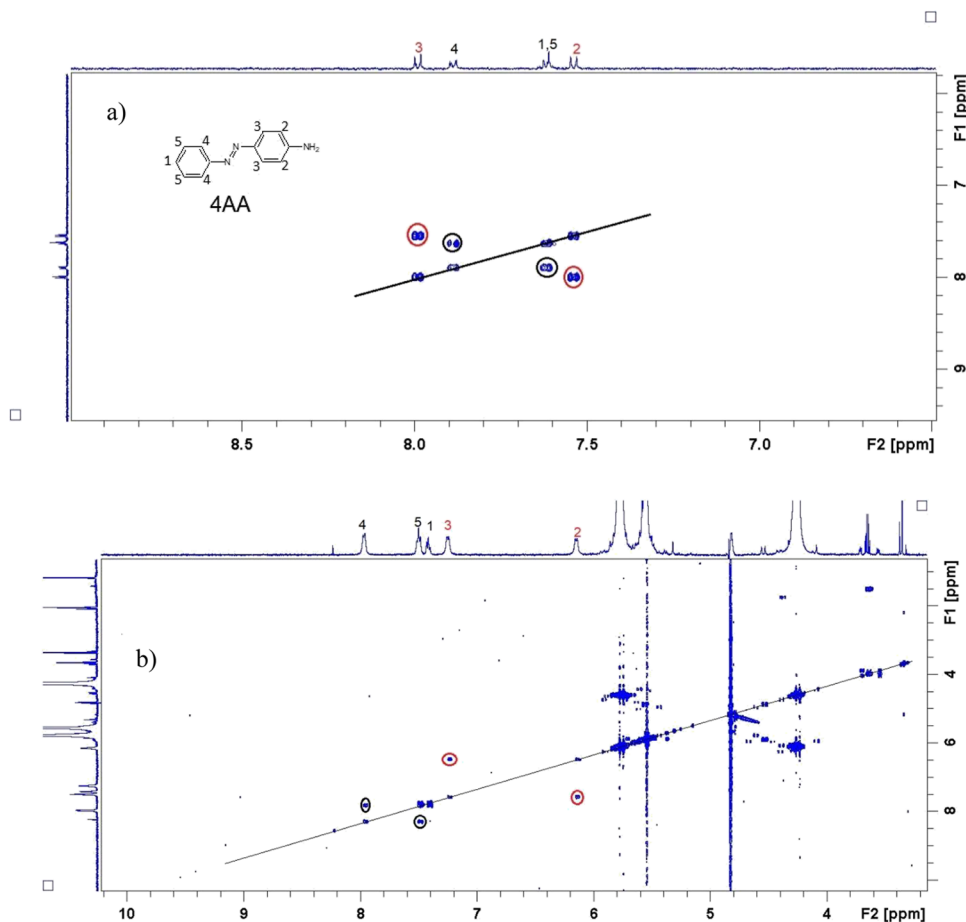
Then, to assign the peaks to the protons of the guest molecule, 2D NMR COSY <sup>1</sup>H-<sup>1</sup>H spectra were carried out at 298 K on free and complexed 4-aminoazobenzene (Figure S4a,b).

We clearly observed a correlation between H3 and H2 (red circle) as well as between H1,5 and H4 (black circle) for the free 4AA (Figure 5a). Likewise, correlations for the most concentrated complex in CB7 combined with the integration of the different peaks led to the assignment of these (Figure 5b).

All the chemical shifts of 4AA protons are reported in Table S1. From these results, we show an upfield shift for both H2 ( $\Delta\delta_{\max} = -1.42$ , where  $\Delta\delta_{\max} = \delta_{\text{bound}} - \delta_{\text{free}}$ ) and H3 protons ( $\Delta\delta_{\max} = -0.77$ ) and also an upfield shift for both the H1 and



**Figure 4.** <sup>1</sup>H NMR spectra in acidic solutions at 298 K of (a) 4AA (0.14 mM), (b–f) mixture CB7/4AA with CB7 increasing concentrations 0.17, 0.34, 0.45, 0.55, and 0.72 mM, (g) CB7 (0.013 mM). 4AA framed in red, CB7 framed in blue, residual water framed in black.

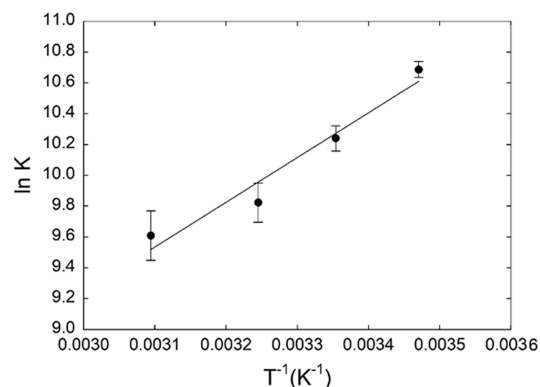


**Figure 5.** 2D NMR COSY  $^1\text{H}$ - $^1\text{H}$  spectrum at 298 K in acidic solutions (pD1) (a) 4AA (b) complex. Correlation between protons circle in red 2 and 3, circle in black 4 and 1,5.

H5 protons ( $\Delta\delta_{\text{max}} = -0.23$  and  $-0.15$ , respectively) even if it is less significant. Consequently, all the protons of the guest experience upfield shifts to varying degrees except protons H4 (showing a slight downfield of  $\Delta\delta_{\text{max}} = +0.05$ ). This situation could be consistent with the entire azobenzene being accommodated within the cavity of CB7 in a twisted form with H4 at its portal.

We have also investigated the thermodynamic properties from the NMR results. Assuming a 1:1 complex, the association constant is calculated at 298 K using a linearization approach<sup>38</sup> derived from Benesi–Hildebrand eq 1.  $^1\text{H}$  NMR spectra in acidic solutions are also performed at various temperatures to obtain the  $\Delta_r H^\circ$  from the van't Hoff relation 3 (Figure 6). All the thermodynamics properties are gathered in Table 1. The thermodynamic properties obtained previously for the association between 4AA and the others host molecules ( $\beta$ -CD, *p*-sulfonatocalix[*n*]arene with *n* = 4,6) are also given in Table 1 for comparison.<sup>27</sup>

As expected, we obtain a significantly larger binding constant with CB7 ( $\log K = 4.9$  from UV spectroscopy and  $\log K = 4.4$  from NMR measurements) than that previously obtained with the other hosts ( $\log K$  around 2.5). Wu et al.<sup>35</sup> studied the association between CB7 and 4,4'-diaminoazobenzene (4DAA) as a function of pH. Interestingly, they obtained a binding constant for the CB7 *trans*-4DAA  $1\text{H}^+$  complex of the same order of magnitude ( $\log K = 5.55$ ) as that obtained in this work.

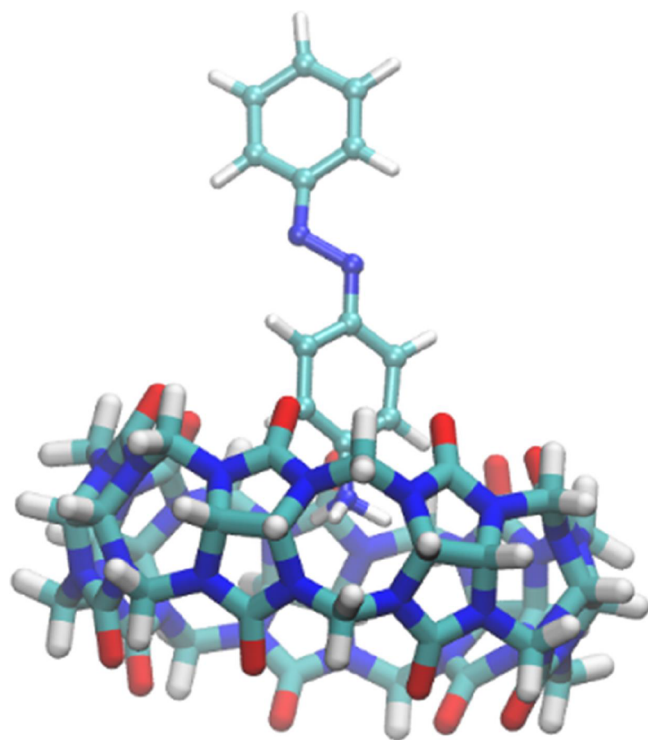


**Figure 6.** van't Hoff plot with  $R^2 = 0.95$ .

In fact, the high binding constant between CB7 and 4AA ( $\log K$  around 5 almost double in comparison with the other host molecules) is the result of a large negative change in  $\Delta_r H^\circ$  ( $-19$  kJ/mol) and a small positive change in  $T\Delta_r S^\circ$  (9 kJ/mol). As observed in Table 1, the binding of all hosts is enthalpy-driven, in line with the insertion of the guest into the host. Nevertheless, the comparison between the  $T\Delta_r S^\circ$  values shows significant differences between calixarenes and both  $\beta$ -CD and CB7. Let us remind that entropic contribution is influenced by two factors, the loss of degree of freedom due to the insertion of the guest within the cavity and the dehydration of the host and guest upon complexation. Concerning

calixarenes, we had previously shown that the negative entropic term was explained by the fact that the flexibility of the cavities was notably reduced.<sup>27</sup> Even if the sign of the entropic term for the CB7 is positive, we note that the change is small (9 kJ/mol). Consequently, we can assume that the flexibility of the CB7 cavity is not significantly reduced and/or that there are few water molecules expelled from the cavity.

In order to verify the encapsulation of 4AA by CB7, knowledge of the precise structure of the complex is required. Molecular modeling should help us further explore these issues. Because it has been shown in the case of free azobenzene in water that the *trans* isomer is thermodynamically more stable than the *cis*-isomer,<sup>51</sup> we first used a force field with an energetic barrier that prevents the *cis*-isomerization for the azobenzene. We first studied the association of CB7 and *trans*-4AA in acidic solutions (Figure 7). The analysis



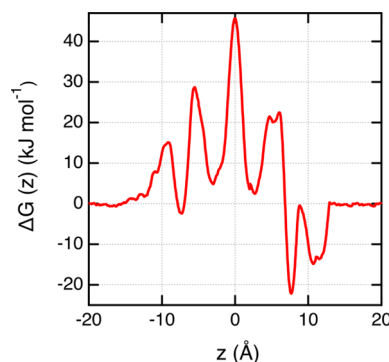
**Figure 7.** Association between CB7 and *trans*-4AA in acidic conditions.

of the equilibrium configuration of *trans*-azobenzene included in CB7 shows that the amine group and the H2 protons are inside the CB7 cavity while the H3 protons of the guest molecule are outside. Interestingly, this equilibrium configuration is not in line with our <sup>1</sup>H NMR results, which pointed out the inclusion of aminoazobenzene within the cavity of CB7.

We have calculated the number of atoms of 4AA inserted into the CB7 cavity. It is around 9, including the amino group, that is, approximately 33% of the molecule. The results<sup>27</sup> previously found with  $\beta$ -CD, *p*-sulfonatocalix[4]arene (C4S), and *p*-sulfonatocalix[6]arene (C6S) were approximately 15, 10, and 16, respectively (55, 37, and 60% of the inserted molecule). Surprisingly, we observe that a few atoms are inserted into the CD's cavity, whereas a large negative change in the  $\Delta_r H^\circ$  value is obtained (Table 1). It is well known that

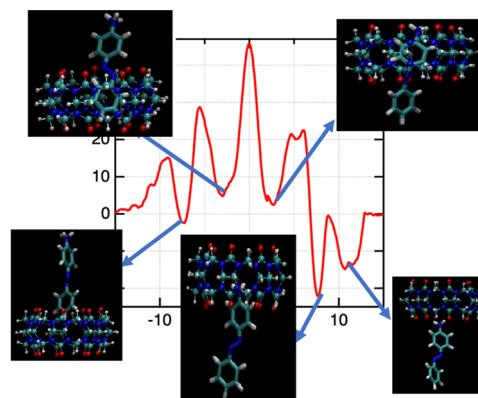
the most favorable  $\Delta_r H^\circ$  is rather associated with the largest number of inserted atoms in inclusion complexation.

Finally, the Gibbs free energy profile or potential of mean force (PMF) for the inclusion of *trans*-4AA into CB7 is shown in Figure 8. The profile shows a central barrier of about 40 kJ



**Figure 8.** Gibbs free energy profile as a function of the *z*-coordinate reaction defined by the separation distance between CB7 and *trans*-4AA molecules.

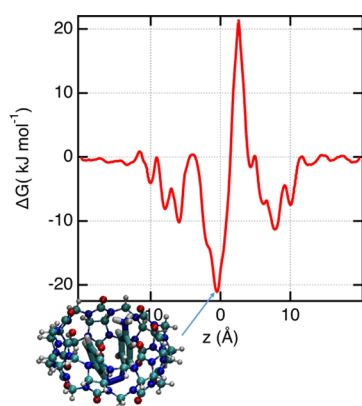
$\text{mol}^{-1}$  flanked by two local positive peaks of about 30 and 20  $\text{kJ mol}^{-1}$ . The deepest Gibbs free energy minimum is located at a separation distance of 7.6 Å with a well depth of  $-22 \text{ kJ mol}^{-1}$ . The second Gibbs free energy minimum of  $-14.3 \text{ kJ mol}^{-1}$  is observed at a distance of about 11 Å. A third, albeit much smaller, is found at a distance of  $-7.5 \text{ Å}$  with a weak well depth of  $-2.3 \text{ kJ mol}^{-1}$ . This curve characterizes the possible conformations of the complex with their associated free enthalpy value (see Figure 9 for the representation of different



**Figure 9.** Typical conformations of the complex with *trans*-4AA at the different free energy minima of the PMF curve.

typical conformations). We observe that the deepest Gibbs free energy minimum located at 7.6 Å corresponds to a partial insertion with the amine group near the carbonyl groups and protons 2 and 3 close to the portal of the host. From Figure 10, the complete encapsulation of 4AA, whereby the benzene rings accommodated within the cavity that correspond to positive free energy minima, can be observed. It means that this conformation for the inclusion of *trans*-4AA into CB7 is not favored from a thermodynamic viewpoint.

Consequently, the only explanation for these deviations from the experimental interpretations is to assume that the complexation induces *trans*-*cis*-isomerization of the 4AA. It should be noted that Wu and Isaacs<sup>35</sup> have already shown the



**Figure 10.** Typical conformations of the complex with *cis*-4AA at the different free energy minima of the PMF curve.

ability of CB7 to drive thermal azobenzene isomerization. To confirm our hypothesis, we calculate the Gibbs free energy profile for the inclusion of 4AA into the CB7 using the *cis*-isomer form of 4AA (*cis*-4AA). The PMF for the inclusion of *cis*-4AA into CB7 is shown in Figure 10. The PMF curve shows only one deeper free energy minimum of  $-21 \text{ kJ mol}^{-1}$  at about  $-0.5 \text{ \AA}$ , indicating that the insertion of *cis*-4AA is possible from a thermodynamic viewpoint.

This complete encapsulation of *cis*-4AA is in perfect agreement with the NMR results.

#### 4. CONCLUSIONS

Detailed NMR and UV studies provided a set of thermodynamic data for the formation of inclusion complexes between 4AA and cucurbit[7]uril in acidic solution. Indeed, the thermodynamic parameters of this complex were determined both by UV spectroscopy and NMR experiments while the structure was specified using NMR and molecular simulations. As expected, a significantly larger binding constant with CB7 ( $\log K$  around 5) than that previously obtained with the other hosts was measured. This larger constant is the result of a large negative change in  $\Delta_r H^\circ$  ( $-19 \text{ kJ/mol}$ ) and a small positive change in  $T\Delta_r S^\circ$  ( $9 \text{ kJ/mol}$ ). The major contribution to the enthalpy of complexation comes from the ion-dipole interaction associated with the inclusion of the 4AA into the CB7 cavity.

Indeed,  $^1\text{H}$  NMR results pointed out the inclusion of the azobenzene within the cavity of CB7. To further understand the experimental results, we also investigated the system by molecular simulations. We first studied the binding interactions of CB7 with *trans*-4AA using a force field that prevents the *cis*-isomerization for aminoazobenzene. However, significant deviations from  $^1\text{H}$  NMR results are obtained, the equilibrium configurations showing only a partial inclusion of 4AA. This led us to hypothesize that the complexation induces *trans*-*cis*-isomerization of the 4AA. Thus, we calculated again the Gibbs free energy profile for the inclusion of 4AA into the CB7 using the *cis*-isomer form of 4AA (*cis*-4AA). From these free energy calculations, we establish that the complete encapsulation of *cis*-4AA is possible from a thermodynamic viewpoint.

The combination of experiments and molecular simulations was powerful to give us insights into the formation and the structure of the complex formed. Nevertheless, the *cis*-*trans* isomerization will require additional methodological develop-

ments to calculate the associated free energy at several separation distances.

#### ■ ASSOCIATED CONTENT

##### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.2c00499>.

Additional UV spectra and  $^1\text{H}$  NMR spectra (Figures S1–S5) as well as Table S1 where chemical shifts are listed (PDF)

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##### Notes

The authors declare no competing financial interest.

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