

## Conformational Dynamics of a Bispyridinium Cyclophane

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**Abstract:** A complete study of the conformational behavior of 4,8-diaza-3(1,4),9(4,1)-dipyridina-1,6(1,4)-dibenzenacyclo-decaphan-3<sup>1</sup>,9<sup>1</sup>-bis(ilium) bishexafluorophosphate is described. This study allows us to conclude that the process observed by which the different chemical shifts of the pyridinium protons show coalescence at a high-temperature <sup>1</sup>H NMR is the rotation around the C–N bond, whereas the conformational equilibrium between the four conformers is produced at low temperature.

The importance of the choline kinase (ChoK) inhibitors as an approach for antiproliferative drug design has been recently reviewed.<sup>1</sup> The synthesis and biological activities of novel bispyridinium cyclophanes (**1–4**, Figure 1) as ChoK inhibitors have been described previously. The ChoK inhibition activities of the cyclophanes strongly depend on the disubstitution model of the upper and lower benzene rings. Compound **4** is the most potent human ChoK inhibitory agent reported to date, showing activity in the low micromolar range.<sup>2</sup>

In addition to their biological activities these novel templates for human choline kinase inhibitors present some very interesting chemical properties. One reason cyclophanes are interesting to NMR spectroscopists is the mobility of the bridges, which is often restricted because of their shortness and therefore brings the rate of existing conformational processes into the range observable by NMR spectroscopy.<sup>3</sup>

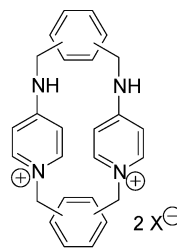
In compound **1** the symmetrical hydrogen atoms of the pyridinium rings show an important difference in chemical shift. The protons H-2 and H-6 of **1**·2PF<sub>6</sub> give rise to two sets of signals ( $\delta$  = 8.36 and 7.85 ppm, DMSO-*d*<sub>6</sub>). Similarly, two sets of resonances are observed for the protons H-3 and H-5 ( $\delta$  = 6.83 and 6.57 ppm, DMSO-*d*<sub>6</sub>).

Conformational analysis of compound **1** has shown four different conformations that are isoenergetic two by two. Figure 2 shows the two energetically different conformations of **1**.

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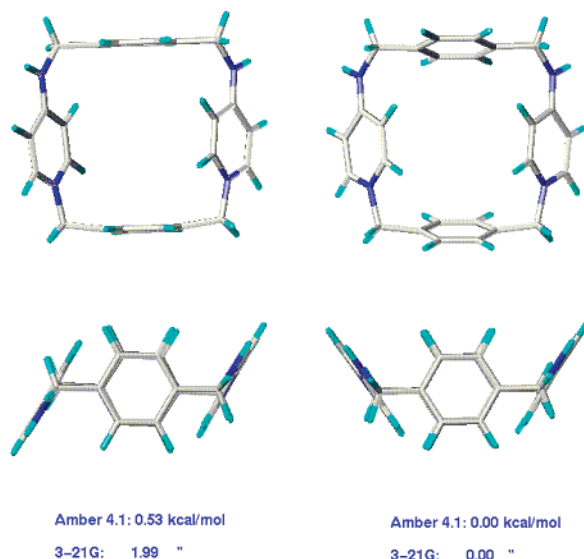
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X = Br for all compounds and moreover PF<sub>6</sub> for **1**

Compound	Isomer
<b>1</b>	<i>p,p</i>
<b>2</b>	<i>m,m</i>
<b>3</b>	<i>p,m</i>
<b>4</b>	<i>m,p</i>

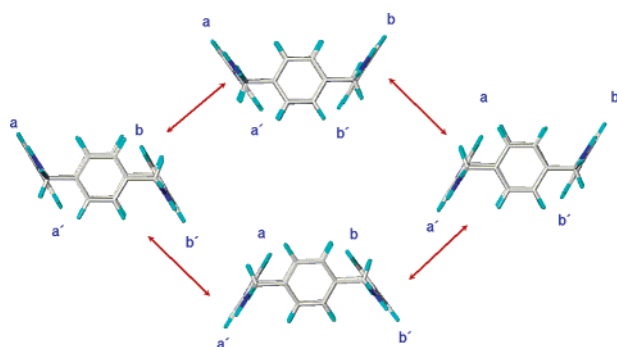
**FIGURE 1.** Structures of cyclophanes **1–4**. The first prefix takes into account the upper linker that connects the amino groups, whereas the second one is related to the disubstitution pattern of the lower benzene ring that links the N<sup>+</sup> atoms.



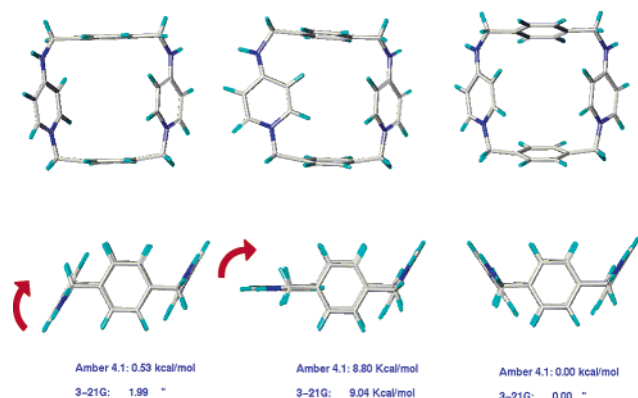
**FIGURE 2.** Side and top views of the two energetically different conformations of compound **1**.

In each of these conformations, one of these protons is orientated toward the center of the two benzene rings, while its symmetrical one points toward the external part of the molecule. Such a different chemical surrounding implies a different shielding that might explain the differences observed in the <sup>1</sup>H NMR spectrum. On the other hand, the NMR high-temperature experiments show an approximation between the pyridinium symmetrical proton signals as the temperature rises, reaching the coalescence temperature at 151 °C (see Supporting Information). Such a coalescence might be explained if the conformational equilibrium that should exist between the four conformers of each compound is taken into account. Such equilibrium is shown in Figure 3, and it can be seen that the relative positions of the pyridinium protons are interchanged as the conformational equilibrium takes place.

To try to explain whether the changes observed in the high-temperature <sup>1</sup>H NMR spectrum were due to this conformational equilibrium, we have calculated the energy of the transition state for the conversion of one conformation into the other one. Such a transition state was located by Gaussian98, using the QST2 keyword and



**FIGURE 3.** Conformational equilibrium between the four conformers of the cyclophane **1**. Relative positions of a and a' or b and b' termini of the pyridinium rings are interchanged during the conformational equilibrium.

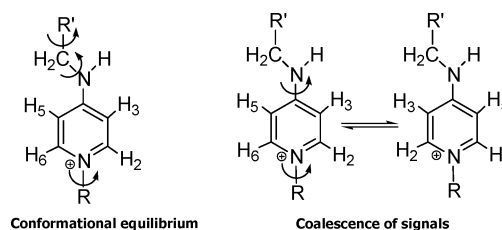


**FIGURE 4.** Transition state for the conversion between the two different conformations found for compound **1**. The indicated Amber 4.1 energy has been obtained by an accurate calculation without optimization of the molecule. A high deformation of the bonding angles centered on the benzylic C atom and the amine N atom is necessary to allow the turn of the pyridinium ring.

the geometries of both conformations as starting and final states, respectively. The character as a transition state was confirmed by calculating the vibrational frequencies of such a conformation, and only one negative was found, indicating that it was a transition state of order 1 (saddle point). Figure 4 shows such a conformation together with its own calculated energies.

The high energy was due to the deformation of the bonding angles centered on the benzylic carbon and on the nitrogen of the amine that is necessary for the turn to take place. Nevertheless, this energy of about 9 kcal/mol is not enough to explain the fact that the coalescence of signals is not produced at temperatures up to 151 °C. On the other hand, the chemical shift differences between the H-2 and H-6 or H-3 and H-5 atoms of the pyridinium ring were 0.51 and 0.26 ppm, respectively. From the coalescence temperature the free energy of activation for the exchange can be calculated. Gutowsky and Holm<sup>4</sup> established a relationship between the coalescence temperature ( $T_c$ ) of two anisocronic groups, difference in chemical shifts ( $\Delta\nu$ ), and the free energy of activation for the exchange. This relation is the following equation:

$$\Delta G_C^\ddagger = 4.57 T_c \left[ 9.97 + \log \frac{T_c}{\Delta\nu} \right] \quad (1)$$



**FIGURE 5.** (Left) Scheme of the rotating bonds during the conformational equilibrium described for compound **1**. (Right) Rotation around the C–N and R–N<sup>+</sup> bonds that would lead to the signal coalescence at 151 °C.

Despite the implicit errors in the equation many approximate measurements of energy barriers have been made by this method. Thus, if the coalescence temperature is 151 °C and if we consider 104 Hz as the chemical shift difference of protons H-3 and H-5 and 204 Hz for H-2 and H-6 the  $\Delta G_C^\ddagger$  calculated values would be 20.5 and 19.9 kcal/mol, respectively. These energies are higher than the 9 kcal/mol value that corresponds to the pseudorotational equilibrium barrier. Accordingly, we can conclude that the coalescence process observed at 151 °C is due to other reasons.

It has been described<sup>5</sup> that the exocyclic C–N internal rotation barrier of the dimethylamino group in the 4-dimethylaminopyrimidine is 12.8 kcal/mol at 247 K, whereas in its hydrochloride form the barrier energy rises to 16.5 kcal/mol at 317 K. This fact is due to the strong double-bond character of C–N as a consequence of the conjugation with the pyrimidinic ring. The above discussion suggests that the rotational barrier should be enhanced if protonation occurs on any of the nitrogen atoms of the pyrimidine ring that carries a dimethylamino group at position 4.

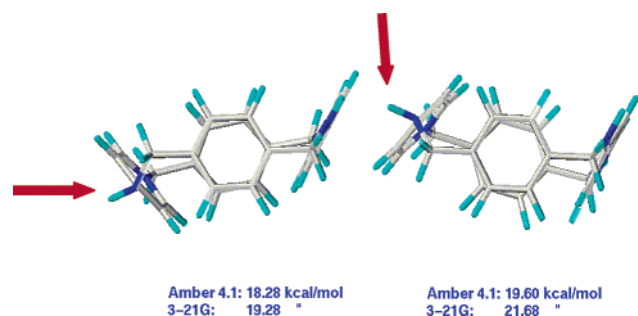
These energy values are in agreement with the ones calculated with eq 1 for bispyridinium cyclophane **1**. That is why we think that the coalescence process observed at 151 °C in **1** has to do with the restricted rotation of the pyridinium ring around the C–N bond rather than with the simple conformational interchange of the molecule.

Figure 5 depicts a scheme of the possible process. In one of the conformations, hydrogen H-3 is “*cis*” (syn-periplanar) in relation to the N–H bond, whereas in the other hydrogen H-5 is the one that occupies such a position. Such a conformational change is distinct from the one that is produced in the above-mentioned conformational equilibrium (Figure 3), in which the pyridinium ring and the amino group act as a block and, accordingly, H-3 is always “*cis*” in relation to the N–H bond.

To confirm that this is the process observed in the <sup>1</sup>H NMR spectrum at 151 °C, the energies of the transition states for the rotation around the C–N and R–N<sup>+</sup> bonds have been calculated using the Gaussian98 program. The keyword QST3 has been employed, needing three geometries for the achievement of the calculations: the initial one, the final one, and a hypothetical one for the transition state where a conformation of **1** has been taken

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**FIGURE 6.** Transition states calculated for the rotation around the C–N and C–N<sup>+</sup> bonds in **1**. The model on the left-hand side has been calculated on the 0.53 kcal/mol conformation, whereas the right-hand one is the transition state calculated in the most stable conformation of the ring. The red arrows show the orientation of the N–H bond, which is perpendicular to the pyridinium ring.

in which a pyridinium ring has been placed perpendicular to the amine N–H bond.

Figure 6 shows the results found for the transition states calculated in the two conformations of **1**. It may be observed that the N–H bond is located in a nearly perpendicular orientation to the pyridinium ring.

The values of these energies, about 20 kcal/mol, are compatible with the barrier calculated by eq 1 for the coalescence of signals at 151 °C due to the rotation of the pyridinium rings.

Finally, we have carried out the low-temperature <sup>1</sup>H NMR experiment trying to observe the conformational interchange of the molecule of about 9 kcal/mol. In fact, at –90 °C (8.5 kcal/mol according to eq 1) an approximation of the signals occurs, it being impossible to lower the temperature further due to the freezing of the solvent (CD<sub>3</sub>OD, mp –98 °C).

Thus, we can conclude that the process observed at high temperature is the rotation around the C–N bond, whereas the conformational equilibrium between the four conformers of **1** would be produced at a low temperature.

## Experimental Section

**4,8-Diaza-3(1,4),9(4,1)-dipyridina-1,6(1,4)-dibenzenacyclodecaphan-3<sup>1</sup>,9<sup>1</sup>-bis(ilium) Dibromide (**1**).** The synthesis of this compound has been previously reported.<sup>2</sup> The compound (**1**) has been named according to IUPAC recommendations for phane structures.<sup>6,7</sup> To improve the solubility of compound **1** we have interchanged the counterions Br<sup>–</sup> for the larger ones PF<sub>6</sub><sup>–</sup>, following the experience of radical solubility of bipyridilium species.<sup>8</sup> Variable-temperature NMR studies have been carried out on the PF<sub>6</sub><sup>–</sup> derivative. The <sup>1</sup>H NMR spectra were recorded in DMSO-*d*<sub>6</sub> for the high-temperature experiments and in CD<sub>3</sub>OD for the low-temperature ones using a spectrometer operating at 400.132 MHz. The center of the peaks of DMSO-*d*<sub>6</sub> (2.50 ppm) and CD<sub>3</sub>OD (3.31 ppm) were used as internal references in a 5 mm <sup>13</sup>C/<sup>1</sup>H dual probe (Wilmad, 528-PP).

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(7) <http://www.chem.qmw.ac.uk/iupac/phane>. Phane Nomenclature. Part II: Substitution Derivatives of Phane Parent Hydrides. IUPAC Recommendations, 2001.

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**Molecular Modeling.** The study of the possible conformations of the cyclophane derivatives has been carried out by the Sybyl<sup>9</sup> program on a Silicon Graphics workstation. The different molecules have been constructed from standard fragments of the libraries of the program, necessitating the definition of a new type of atom (N.ar4) for the quaternary nitrogen of the pyridinium fragments. The force field Amber 4.1,<sup>10</sup> implemented in the Sybyl program, has been used in the energy calculations. For the pyridine nitrogen the atomic N\* type has been used, corresponding to a nitrogen with sp<sup>2</sup> hybridization, needing the definition of new parameters (Supporting Information available), which were generated by ab initio calculations on model molecules.

Once the initial geometries were generated, we proceeded to their optimization using the Powell<sup>11</sup> method. The atomic charges were calculated by means of the AM1<sup>12</sup> Hamiltonian implemented in the MOPAC 6.0<sup>13</sup> program. A distance-dependent dielectric constant with a value of  $\epsilon = 1$  was used, and the optimization was continued until the energy gradient was less than 0.01 kcal/mol·Å<sup>2</sup>. Conformational searches were carried out by means of molecular dynamics, using the “simulated annealing” technique, heating the molecule up to 1000 K for 1000 ps, cooling it down later exponentially to 200 K, and maintaining it for another 1000 ps. Five hundred heating–cooling cycles were carried out on each molecule, and the geometries obtained at the end of each cooling period were kept. These 500 conformations were optimized under the same conditions described before and were compared with each other to remove those that were geometrically and energetically equal.

In this way, four conformations for compound **1** were identified. Such conformations were optimized using ab initio (3-21G) calculations using the Gaussian98<sup>14</sup> program. After this, those conformations that were energetically and geometrically different were selected for their subsequent study.

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**Supporting Information Available:** High- and low-temperature NMR experiments and calculated parameters used in the Amber 4.1 force field. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO0301292

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