Solution structure and conformational equilibria of a symmetrical calix[6]arene. Complete sequential and cyclostereospecific assignment of the low-temperature NMR spectra of a cycl...

ARTICLE *in* THE JOURNAL OF ORGANIC CHEMISTRY · JUNE 1992

Impact Factor: 4.72 · DOI: 10.1021/jo00051a046

6 AUTHORS, INCLUDING:

Pedro M Nieto
Spanish National Research Council
96 PUBLICATIONS **2,156** CITATIONS
SEE PROFILE

Concha Sanchez-Martinez
Eli Lilly
28 PUBLICATIONS **803** CITATIONS
SEE PROFILE

Miquel Pons
University of Barcelona
177 PUBLICATIONS **3,289** CITATIONS
SEE PROFILE

(3:1) (500 mL × 5) at room temperature, and the combined extracts were evaporated in vacuo to give an aqueous phase, which was extracted with EtOAc. Evaporation of the combined EtOAc extracts afforded 23 g of a crude organic extract, which was separated by MPLC on a SiO_2 column using sequential mixtures of petroleum ether and EtOAc as eluants.

Isolation of Lintenone. Fractions eluted with petroleum ether/EtOAc (4:6) afforded a mixture of 980 mg containing lintenone. Its purification was achieved by HPLC using a Hibar LiChrospher Si60 (7-μm) column with a mobile phase of *n*-hexane/EtOAc (7:3).

Lintenone: yield 253 mg; $[\alpha]^{25}_D = -75.5°$ (*c* 0.004, $CHCl_3$); $[\theta]_{300} = -9574$ (EtOH); IR = 1783, 1749, 1714 cm^{-1} (KBr); ^1H and ^{13}C NMR spectra see Tables I and II; HREIMS (70 eV) obsd *m/z* 384.2666, $C_{25}H_{36}O_3$, calcd *m/z* 384.2666.

Acknowledgment. This work is a result of research supported by CNR, Progetto Finalizzato Chimica Fine II, and by MURST Rome, Italy. We wish to thank Prof. W. Fenical for giving us the opportunity to participate in an expedition to the Caribbean Sea, during which the sponge *C. cf. linteiformis* was collected. We are grateful to Dr. G. Villani, Istituto per la Chimica di Molecole di Interesse Biologico, CNR, Arco Felice, Italy, for the antifeedant and ichthyotoxicity tests. We thank Mr. G. Scognamiglio, Istituto per la Chimica di Molecole di Interesse Biologico del CNR, Arco Felice, Italy, for the CD spectrum. NMR and IR spectra were performed at "Centro Interdipartimentale di Analisi Strumentale", Università di Napoli Federico II.

Supplementary Material Available: 1D and 2D NMR spectra and a CD spectrum of lintenone (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Solution Structure and Conformational Equilibria of a Symmetrical Calix[6]arene. Complete Sequential and Cyclostereospecific Assignment of the Low-Temperature NMR Spectra of a Cycloasymmetric Molecule

M. Antônia Molins,† Pedro M. Nieto,‡ Concha Sánchez,‡ Pilar Prados,‡ Javier de Mendoza,‡ and Miquel Pons*,†

Departament de Química Orgânica, Facultat de Química, Universitat de Barcelona, 08028-Barcelona, Spain, and Departamento de Química, Universidad Autónoma de Madrid, Cantoblanco, 28049-Madrid, Spain

Received June 18, 1992

A calix[6]arene containing *tert*-butyl and chlorine substituents in alternate rings, with a 3-fold sequential symmetry, could be frozen in a completely asymmetrical conformation at 183 K in CD_2Cl_2. The ^1H-NMR spectrum could be completely assigned by low-temperature ROESY, DQF-COSY, HMQC, and HMBC experiments using a sample in which the phenolic protons had been partially exchanged by deuterons to reduce both spin diffusion and conformational exchange processes. The three-dimensional structure obtained using restrained molecular dynamics is a winged cone made asymmetric by the clockwise or anticlockwise sense of a cyclic array of hydrogen bonds. Three different types of exchange processes, leading to a statistically symmetric conformation at room temperature, could be identified in the NOESY and ROESY experiments. Hexa-*tert*-butylcalix[6]arene, with a potential 6-fold symmetry, seems to have a very similar conformation at low temperature with a C_2 axis as the only symmetry element.

Introduction

One of the major goals of supramolecular chemistry is the design of receptors for cationic, anionic, or neutral organic substrates. A large number of macrocyclic and cleftlike structures have been designed for this purpose.[1] Calix[4]arenes, the cyclic tetramers of phenols linked by methylene groups between the ortho positions, have attracted particular attention for this issue because they can adopt conformations (cone, partial cone) that contain a cavity.[2] However, due to their small size, inclusion complexes are not easily formed in solution,[3] and calix[4]arenes are now better viewed as molecular platforms on which functional groups can be oriented in space to define cavities or clefts.[4]

The cavities of calix[6]arenes, the cyclic hexamers of phenols, are larger, and therefore they show better prospects for the formation of inclusion complexes or channels. These substances are much more flexible, and they can adopt a number of conformations. Proper functionalization in the upper or lower rim can be envisaged as a mean of controlling the conformation and, therefore, modulating the properties of calix[6]arenes. For example, lower rim hexa-O-substituted derivatives of hexa-*tert*-butylcalix[6]arene (1)[5] have been reported for the development of ionophores selective for uranyl,[6] alkali and metal,[7] and

† Universitat de Barcelona.
‡ Universidad Autónoma de Madrid.

(1) See, for example: (a) *Top. Curr. Chem.* **1981**, *98*; **1982**, *101*; **1984**, *121*; **1985**, *128*; **1986**, *132–136*; **1987**, *140*; **1988**, *149*. (b) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1009–1112. (c) Lehn, L.-M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304–1319. (d) Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245–255. (e) Nowick, J. S.; Feng, Q.; Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8831–8839.

(2) (a) Gutsche, C. D. *Calixarenes, Monographs in Supramolecular Chemistry*; Stoddart, F. J., Ed.; Royal Soc. Chem.: Cambridge, 1989; Vol. 1. (b) *Calixarenes, A Versatile Class of Macrocyclic Compounds*; Vicens, J., Böhmer, V., Eds.; Kluwer: Dordrecht, 1990.

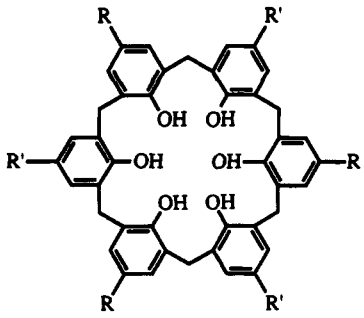
(3) (a) Andreetti, G. D.; Ungaro, R.; Ponchini, A. *J. Chem. Soc., Chem. Commun.* **1979**, 1005–1007. (b) Bott, S. G.; Coleman, A. W.; Atwood, J. L. *J. Am. Chem. Soc.* **1986**, *108*, 1709–1710. (c) McKervey, M. A.; Seward, E. M.; Ferguson, G.; Ruhl, B. L. *J. Org. Chem.* **1986**, *51*, 3581–3584.

(4) van Loon, J.-D.; Heida, J. F.; Janssen, R.; Verboom, W.; Reinhoudt, D. N. *16th International Symposium on Macrocyclic Chemistry*, Sheffield, 1991.

(5) Gutsche, C. D.; Dhawan, B.; Leonis, M.; Stewart, D. *Org. Synth.* **1989**, *68*, 238–242.

(6) Nagasaki, T.; Shinkai, S.; Matsuda, T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2617–2618 and references cited therein.

0022-3263/92/1957-6924$03.00/0



1 R = R' = t-Bu
2 R = Cl; R' = t-Bu

ammonium[8] cations, but just a few partially O-substituted derivatives have been described.[9] At the upper rim, the only known derivative without the same substituents at the para positions is calix[6]arene 2 (5,17,29-tri-*tert*-butyl-11,23,35-trichlorocalix[6]arene), with alternate chlorine and *tert*-butyl groups.[10]

The possible conformations of calix[6]arenes in solution have been classified by Gutsche into two families called "hinged" (with three contiguous aryl groups "up" and the other three "down") and "winged" (two aryl groups in "out" alignments and the other four in "up" and/or "down" positions).[11] X-ray data for 1-benzene (1:3) complex[12] as well as for free *p*-isopropylcalix[6]arene[12] and free 1[13] support winged cone structures for these molecules in the solid state.

In order to define completely the conformational properties of a flexible molecule in solution one should determine the structure of the different conformations and characterize the interconversion pathways.

Solid-state structures may not be appropriate models for the conformations of flexible molecules because of the effect of solvent or packing forces. Furthermore, no information is provided on the dynamic behavior associated with a given structure. NMR is now an established method for obtaining three-dimensional structures for rigid molecules in solution, and it has been most successful in the study of biological molecules.[14] Furthermore, NMR has been used for many years to study the dynamics of conformational interconversions in flexible molecules.

We have determined the three-dimensional structure of 2 at low temperature in solution by NMR. In our conditions all the conformational equilibria are frozen and a single conformation is present. This conformation is asymmetric in spite of the potential C_3 symmetry of the molecule because of the sense of rotation in the cyclic array of hydrogen bonds that stabilize the structure.

The complete sequential and stereospecific assignment of the NMR spectrum of this asymmetric conformation has allowed us to define the topoisomerization processes responsible for the average symmetry observed at room temperature and measure the corresponding interconversion barriers. This study provides a complete model for the conformation of a flexible calix[6]arene in solution and describes a general methodology that could be applied to the study of other flexible molecules in solution.

Table I. Symmetry Dependence of the Expected Proton Spectra for the Hexa-*tert*-butylcalix[6]arene

symmetry element	group intersected by the element	phenolic[a] protons	methylene[a] protons	*tert*-butyl[a] protons
C_2 axis eq	methylenes	three singlets	two AX + two A_2	three singlets
C_2 axis eq	aromatic rings	four singlets	three AX	four singlets
C_2 axis ax	–	three singlets	three AX	three singlets
plane	methylenes	three singlets	four AX	three singlets
plane	aromatic rings	four singlets	three AX	four singlets
center	–	three singlets	three AX	three singlets
obsd	–	three singlets	three AX	three singlets

[a] Maximum number of expected signals for each type of proton (accidental degeneracy can lower the observed number).

Results and Discussion

Comparison of 1 and 2. ^{1}H-NMR spectra of 1 and 2 in $CDCl_3$ were recorded at temperatures from 293 to 223 K. The spectra at 223 and 293 K of both compounds are compared in Figure 1. At room temperature the spectrum of 1 contains only four signals due to fast conformational average indicating that the six phenol rings are equivalent on the NMR time scale. The spectrum of 2 contains six singlets due to the presence of two types of phenolic rings.

At 223 K, the spectrum of 1 contains separate signals from three different phenolic protons, three AX systems from the methylene groups and three nonequivalent *tert*-butyl and aromatic groups. At this temperature, protons in positions 3 and 5 of each aromatic ring are not equivalent and the aromatic region consists of six signals as the coupling is not resolved. This is in agreement with the results of Gutsche[11] obtained at a lower field strength. In compound 2 the lower symmetry results in a doubling of signals. At 223 K its ^{1}H-NMR spectrum displays six nonequivalent phenolic protons and two singlets of relative intensity 2:1 in the *tert*-butyl region. The aromatic region obtained from traces of an HMQC experiment, due to the complexity of the 1D spectrum, shows evidence for 12 distinct protons.

The methylene region contains six pairs of doublets which can be grouped into two slightly shifted sets of three. Each of these sets shows approximately the same chemical shifts and chemical shift differences between coupled pairs of protons found in 1. This was confirmed with a double quantum filtered COSY experiment at 223 K (Figure 2) where five of the six possible cross-peaks are resolved and they are clearly grouped in pairs.

The similarities between the methylene regions of 1 and 2 suggest that at low temperature both compounds have the same conformation, and the differences arise mainly because a binary symmetry element (axis, center, or plane) present in 1 is destroyed by substituting a chlorine for a *tert*-butyl group in alternate positions. Table I shows the expected spectral characteristics of 1 depending on the symmetry element present in its low-temperature con-

(7) Arnaud-Neu, F.; McCollins, E.; Deasy, M.; Ferguson, G.; Harris, S. J.; Kaitner, B.; Longh, A. J.; McKervey, M. A.; Marques, E.; Ruhl, B. L.; Schwing-Weill, M. J.; Seward, E. M. *J. Am. Chem. Soc.* **1989**, *111*, 8681–8691.

(8) Chang, S. K.; Hwang, H. S.; Sau, M.; Youk, J.; Kang, S. *J. Chem. Soc., Chem. Commun.* **1991**, 217–218.

(9) For a 1,2,4,5-tetra-*p*-nitrobenzoate of 1, see: Gutsche, C. D.; Rogers, J. S.; Stewart, D.; See, K. A. *Pure Appl. Chem.* **1990**, *62*, 485–491. The 1,2,4,5-tetrapicolyl derivative of 1 has also been prepared: Neri, P.; Foti, M.; Ferguson, G.; Gallagher, J.; Kaitner, B.; Pons, M.; Molins, M. A.; Giunta, L.; Pappalardo, S. *J. Am. Chem. Soc.* **1992**, *114*, 7814–7821. For a 1,3,5-trimethyl ether, see: Casnati, A.; Minari, P.; Pochini, A.; Ungaro, R. *J. Chem. Soc., Chem. Commun.* **1991**, 1413–1414.

(10) de Mendoza, J.; Nieto, P. M.; Prados, P.; Sánchez, C. *Tetrahedron* **1990**, *46*, 671–682.

(11) Gutsche, C. D.; Bauer, L. J. *J. Am. Chem. Soc.* **1985**, *107*, 6052–6059.

(12) Halit, M.; Oehler, D.; Perrin, M.; Thozet, A.; Perrin, R.; Vicens, J.; Bourakhouadar, M. *J. Incl. Phenom.* **1988**, *6*, 613.

(13) Andreetti, G. D.; Calestani, G.; Ugozzoli, F.; Arduini, A.; Ghidini, E.; Pochini, A.; Ungaro, R. *J. Incl. Phenom.* **1987**, *5*, 123–126.

(14) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; J. Wiley & Sons: New York, 1986.

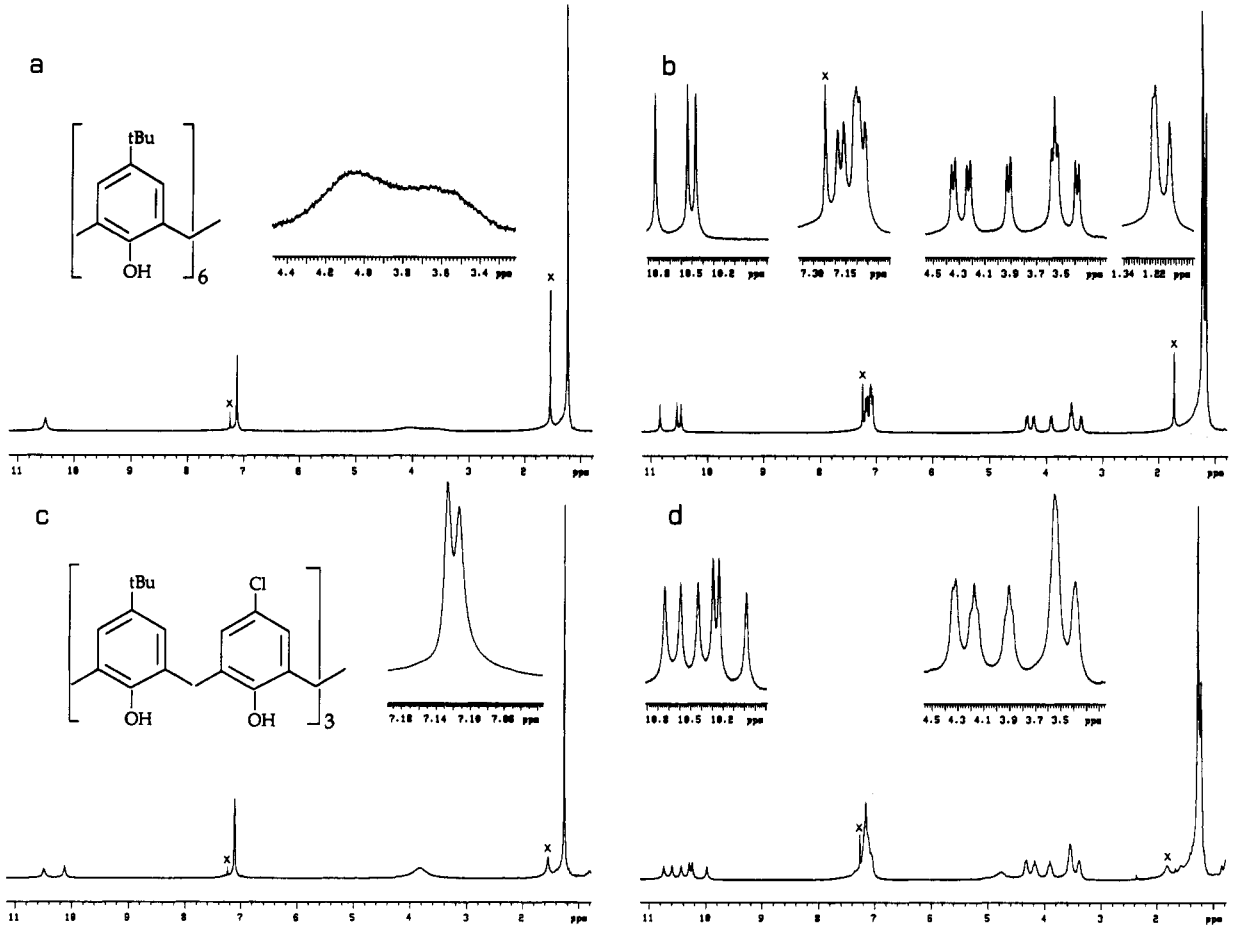


Figure 1. One-dimensional spectra of hexa-*tert*-butylcalix[6]arene (1) in $CDCl_3$ at (a) 293 K and (b) 223 K and of **2** at (c) 293 K and (d) 223 K. Signals marked with x are from $CDCl_3$ and water.

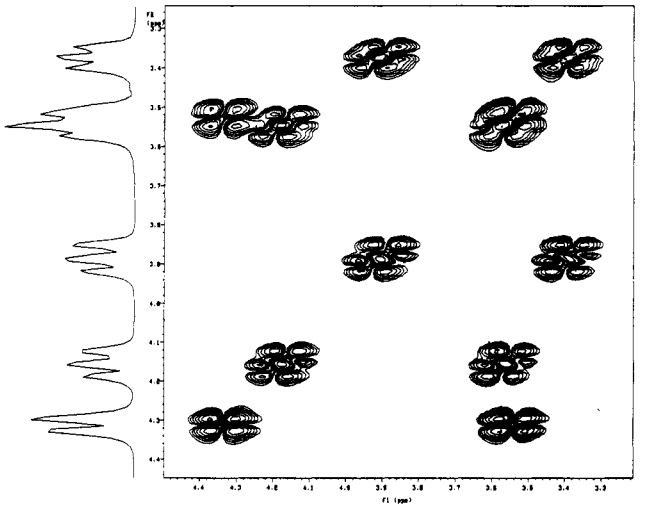


Figure 2. Methylene region of a DQF-COSY experiment of **2** in $CDCl_3$ at 223 K. The large signal at 3.56 ppm accounts for four nearly degenerate protons. Assignments are given in Table II and Figure 5.

formation. From this analysis one must conclude that, at low temperature, **1** contains either a C_2 axis perpendicular to the mean plane of the macrocyclic ring or a center of symmetry.[15]

(15) In the case that the symmetry element were a C_2 axis perpendicular to the mean plane of the macrocyclic ring the conformation would be chiral, and in fact two mirror-image conformations would exist.

Sequence-Specific Assignment of 2. The 1D spectrum of **2** in $CDCl_3$ at 223 K shows well-resolved resonances for nearly all protons. At this temperature and in this solvent resonances belonging to the same phenolic or bridging methylene unit could be identified, and each methylene resonance could also be assigned to a proton pointing toward the upper or lower rim. Nevertheless, analysis of the 2D-NOESY and ROESY spectra (see below) at that temperature revealed that considerable conformational exchange was still present. This complicated the analysis considerably and, in particular, prevented the measurement of short distances between protons belonging to neighbor units that could allow a complete sequence specific assignment of the spectra.

In CD_2Cl_2 at 223 K the 1D spectrum shows broad lines indicating that in this solvent the conformational exchange is faster than in $CDCl_3$. At 183 K in CD_2Cl_2 the 1D spectrum contains sharp lines but a ROESY experiment at 183 K using a mixing time of 200 ms still gives weak exchange cross-peaks in the methylene region, between two of the sites, and in the phenolic region, where protons exchange in pairs. While the ROESY cross-peaks between methylenic and phenolic protons are now well-resolved, giving specific interactions that allow the correlation of each methylene group with the phenolic proton of one of its flanking rings, no cross-relaxation cross-peaks could be observed between phenolic protons. This is a surprising result as ROE interactions between these protons were present in $CDCl_3$ at 223 K. A possible explanation for this observation is an enhancement of spin diffusion at the lower temperature causing a reduction of the intensity of the cross-peaks. An alternative explanation is a mutual

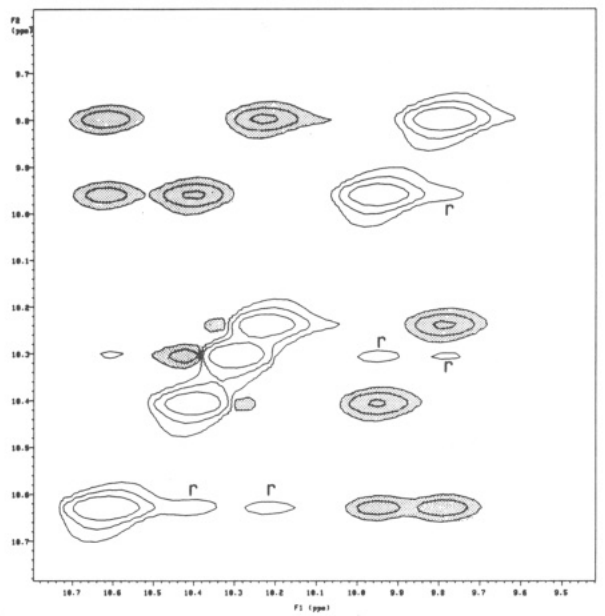


Figure 3. ROESY spectrum of a partially deuterated sample of **2** in CD_2Cl_2 at 183 K (t_{mix} = 200 ms). Positive cross-peaks (labeled r) are very weak and probably come from three spin effects. Strong negative cross-peaks correlate each phenolic proton to the ones belonging to flanking rings. The lower intensity of the cross-peaks close to the diagonal is due to partial cancellation because of the different signs. Assignments are given in Table II and Figure 5.

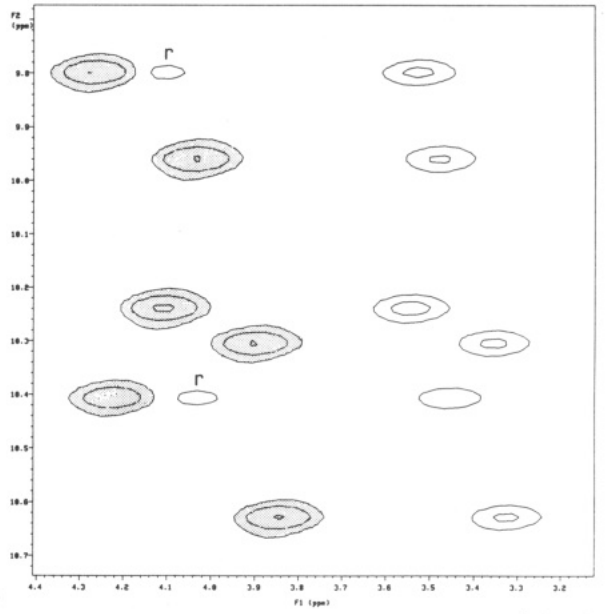


Figure 4. Expansion of a ROESY spectrum of a partially deuterated sample of **2** in CD_2Cl_2 at 183 K showing the connectivities between phenolic and methylene protons. Negative cross-peaks indicate direct cross-relaxation. Positive cross-peaks with the methylene protons at high field arise from composite TOCSY–NOESY pathways. The two weak positive peaks labeled r come from three spin effects. Assignments are given in Table II and Figure 5.

cancellation of cross-peaks arising from cross-relaxation and exchange because of their different sign.

Both effects could be eliminated by partially exchanging the hydroxy protons by deuterons. Deuterium nuclei, having a much lower gyromagnetic ratio than protons, provide a much less efficient relaxation pathway, and cross-relaxation among the remaining protons is enhanced. Furthermore, isotopic effects are expected for conformational changes involving species stabilized by hydrogen bonds[16] and also for hydrogen exchange, especially if proton tunneling plays a significant role in the process.[17]

At 183 K the 1D ^{1}H-NMR spectrum of a ~50% deuterated sample shows sharp well-resolved lines for all the protons, and the phenolic region of the ROESY spectrum at this temperature shows strong, well-resolved cross-relaxation cross-peaks (Figure 3) correlating each of the six protons to two other phenolic ones. Each phenolic proton shows a strong negative cross-peak arising from cross-relaxation with only one methylene proton (Figure 4). Each phenolic proton also shows a positive cross-peak with one of the high-field methylenes. These probably arise from a combined TOCSY–NOESY pathway. The weak positive cross-peaks labeled *r* in Figure 4 most probably arise from relayed coherence transfer $OHOHCH_2$ (three-spin effect). This is consistent with the fact that the direct cross-relaxation between the two hydroxy groups involved is very effective as seen from the intensity of the corresponding cross-peak. Weaker positive signals, visible at a higher vertical scale, can be explained in the same way although through less effective pathways.

The whole set of connectivities can be mapped in a hexagonal graph containing the six phenolic protons. This arrangement explains the fact that each phenolic proton cross-relaxes with two others and is consistent with a circular array of hydrogen bonds as shown in Figure 5. This allows the sequential assignment of the phenolic protons but does not discriminate between the different possibilities arising from the cyclic nature of this system.

In Figure 5, **a** and **b** represent two mirror image arrangements of the hydroxy protons consistent with the experimentally detected nearest neighbor relationships and **c** and **d** show two possible arrays of hydrogen bonds which are also enantiomeric. The correct assignment amounts to choosing between the possible combinations of both arrangements: **ac**, **bd**, **ad**, and **bc**. Combinations **ac** and **bd** would be the assignments of enantiomeric conformations and cannot be differentiated. The same is true for combinations **ad** and **bc** but combinations **ac** and **ad** give two different assignments. We believe that the correct one is **ac** or its mirror image and the corresponding assignment is given in **e**. The reasons for this selection will become clear when we discuss the three dimensional structure and dynamic behavior of **2**.

This assignment can be extended (Figure 5e) to the low-field methylene protons using the direct correlation cross-peaks of the ROESY spectra to the high-field methylene protons using the TOCSY–NOESY cross-peaks.

Having established the sequence specific assignment, the last problem to be solved concerns the two possible arrangements of the chlorine and *tert*-butyl substituents.

Assignment of the Substitution Pattern. In order to solve this problem a connectivity had to be established between the phenolic protons and the *tert*-butyl substituents. Considering the symmetry of our system the only unambiguous way of finding this correlation was through scalar coupling along the aromatic rings which implies the assignment, at least partially, of the ^{13}C-NMR spectrum of **2**. In the *tert*-butyl-substituted rings carbon 4 could be distinguished by the three-bond coupling to the methyl protons and carbons 3 (5) could be identified by the coupling to the quaternary carbon of the *tert*-butyl group. From this carbon a one-bond connectivity allowed the

(16) While this manuscript was in preparation a report has appeared indicating a strong isotopic effect in the conformational interconversion of calix[4]arenes with carboxilic or carboxamide substituents in the upper rim: Conner, M.; Janout, V.; Regen, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 9670–9671.

(17) Bell, R. P. *The Tunnel Effect in Chemistry*; Chapman and Hall: Cambridge, 1980.

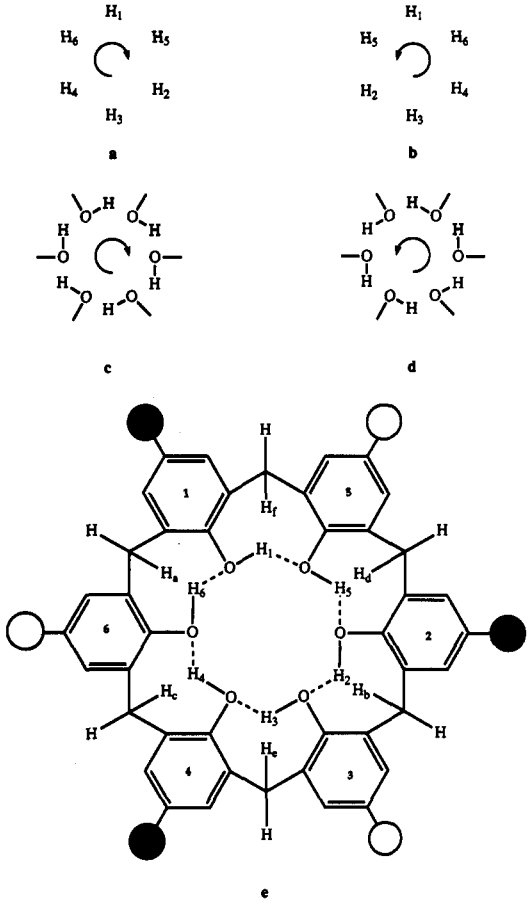


Figure 5. Sequential assignment of 2 based on the connectivities established in a ROESY experiment using a partially deuterated sample of 2 in CD_2Cl_2 at 183 K. Phenolic protons are labeled with arabic numerals in order of decreasing frequency. Low-field methylene protons are labeled with small letters in alphabetical order of decreasing frequency. The coupling partners of the methylene protons appear at higher field and are labeled with the corresponding capital letter. In CD_2Cl_2 at 183 K the frequencies of these protons follow the order C > A > D > B > E > F. Note that in the actual molecule these hexagonal arrays of atoms can not be planar if the HOH angles of the hydrogen bonds take their expected values. a and b show two arrangements of the phenolic protons compatible with the neighborhood relations determined experimentally. b and c show the two possibilities for the cyclic hydrogen bond array. The final assignment is shown in e. The question of which phenolic protons belong to *tert*-butyl-substituted rings and which to the chlorine-substituted ones was solved independently using HMBC and HMQC experiments with the same sample and experimental conditions. Shaded circles represent chlorine atoms and the open circles the *tert*-butyl groups.

identification of the corresponding aromatic protons, and a three-bond coupling of these protons afforded the assignment of carbon 1, ipso to the phenolic group. At 183 K in CD_2Cl_2 using the same partially deuterated sample that had allowed the sequential assignment of the proton spectra, the six phenolic protons could be correlated to six aromatic carbons via a three-bond coupling and two of the protons showed a two-bond coupling to their ipso carbons that finally allowed the identification of the two sets. Numbered in order of decreasing frequency the phenolic protons 1, 2, and 4 correspond to chlorine-substituted rings and protons 3, 5, and 6 to the *tert*-butyl substituted ones. The final assignment is shown in Figure 5e and the proton chemical shifts are presented in Table II.

Three-Dimensional Structure of 2 at Low Temperature in Solution. The three-dimensional structure of 2 at 183 K was obtained using restrained molecular dynamics and is shown in Figure 6.

Table II. Proton Chemical Shifts of Compound 2 in CD_2Cl_2 at 183 K

subst	no. of OH[a]	δ OH[b]	no. of CH_2[a]	δ CH_2	δ Ar	δ CH_3
Cl	1	10.63	f/F	3.86/3.34	7.09/7.19	
tBu	5	9.96	d/D	4.04/3.48	7.11/7.17	1.22
Cl	2	10.40	b/B	4.23/3.47	7.04/7.05	
tBu	3	10.30	e/E	3.90/3.37	7.12/7.19	1.22
Cl	4	10.23	c/C	4.12/3.56	7.16/7.16	
tBu	6	9.80	a/A	4.28/3.54	7.04/7.04	1.14

[a] In each column protons are listed following the sequential order in the molecule clockwise. The labeling of phenolic and methylene protons is defined in Figure 5. [b] Chemical shifts are in parts per million downfield from TMS.

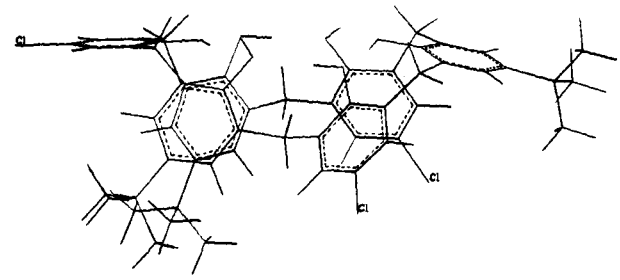


Figure 6. Drawing of a three-dimensional structure obtained by restrained molecular dynamics using the data obtained in CD_2Cl_2 at 183 K. Note that this is a chiral structure and its mirror image, which differs in the sense of rotation of the cyclic array of hydrogen bonds, is also a valid solution.

The low-temperature conformation of 2 is a winged cone with four aryl groups in "up" alignment and two aryl groups located in opposite positions across the ring, bent outside ("out"). In this conformation one expects that the phenol protons of the two "out" rings and the methylene groups flanked by two "up" aromatic rings will be different from the rest. Hydroxy protons 2 and 6 (see Figure 4) and methylene groups e and f (see Figure 7) show distinct cross-relaxation or exchange behavior, thus providing support to the final assignment in Figure 5e with rings 2 and 6 bent outside.

This conformation is stabilized by a cyclic array of hydrogen bonds. The symmetry of this structure when only the backbone of aromatic and methylene groups is considered contrasts with the lack of any element of symmetry if the substituents and hydroxy groups are also considered. This explains the observation of separate signals for each proton and carbon atoms in the low-temperature NMR spectra. In the spectra of 1, in which there is only one type of substituent, the number of signals is only reduced to one half of those observed in the spectra of 2. Therefore, it is clear that the main reason for the lack of symmetry of 1 and 2 is the directionality of the hydrogen bonds in the cyclic array. Going from donor to acceptor in each hydrogen bond the cycle can be followed either in a clockwise or counterclockwise manner. These two possibilities give rise to two enantiomeric[18] winged cone conformations that interconvert slowly at 223 K in $CDCl_3$. Given the ability of the calixarenes to form noncovalent complexes, it would be interesting to investigate the possibility of forming diastereomeric complexes, involving the two cycloenantiomeric conformations, using a chiral guest.

(18) For a discussion of cycloisomerism see: Prelog, V.; Gerlach, H. *Helv. Chim. Acta* **1964**, *47*, 2288–2294. The term *conformational cycloenantiomerism* has been suggested for the cases in which the ring directionality is given by the relative conformational orientation of nonbonded atoms: Singh, M. D.; Siegel, J.; Biali, S. E.; Mislow, K. *J. Am. Chem. Soc.* **1987**, *109*, 3397–3402.

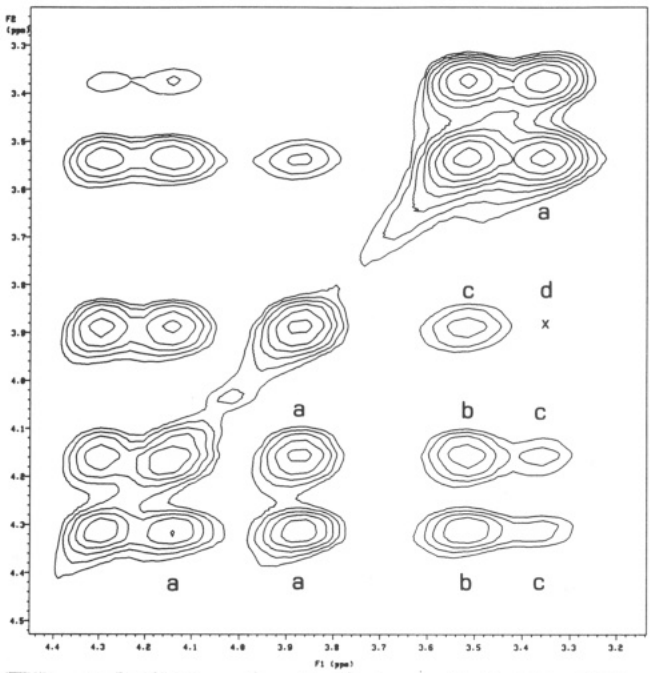


Figure 7. ROESY spectrum (methylene region) of **2** in $CDCl_3$ at 223 K (mixing time 200 ms). All peaks are positive. Peaks labeled a and b correspond to the two exchange processes mentioned in the text. Peaks labeled c can be explained by a combination of the two processes. At longer mixing times a cross-peak appears at the position labeled d. This corresponds probably to a three-step exchange.

The chemical shift of the bridging methylene carbons has recently been suggested as a conformationally diagnostic feature in the calix[4]arene series.[19] A chemical shift around 31 ppm corresponds, in that series, to a syn orientation of the adjacent rings, while a chemical shift around 37 ppm indicates an anti orientation. Although the validity of this rule has not yet been proved in the calix[6]arene series existing evidence in a number of differently substituted compounds indicates that the methylene carbon chemical shifts also tend to have one of two possible values (ca. 31 and ca. 37–39) and that the latter appears in cases where independent evidence indicates the presence of an anti orientation of two adjacent rings[20] although it should be emphasized that, in the flexible calix[6]arenes at room temperature, the chemical shifts just reflect the average conformation. The syn average orientation predicted by the application of the above rule to the room temperature spectra of **1** and **2** is consistent with the three-dimensional structure of **2** found at 183 K.

Conformational Flexibility of 2. At 293 K both **1** and **2** show average conformations of high symmetry characterized by a singlet resonance for all the methylene protons. At 223 K in $CDCl_3$, although clearly below the coalescence temperature, the NOESY and ROESY experiments are dominated by exchange effects.

Exchange cross-peaks in the low-temperature ROESY spectra give information on the conformational flexibility of **2**.

The ROESY experiment at 223 K allows the phenolic protons to be grouped into two sets of three (Figure 8). All the exchange cross-peaks that correlate the three protons of each set have the same intensity, indicating a rapid scrambling among the three sites. Each group corresponds to one of the chemically distinct types of phenolic protons

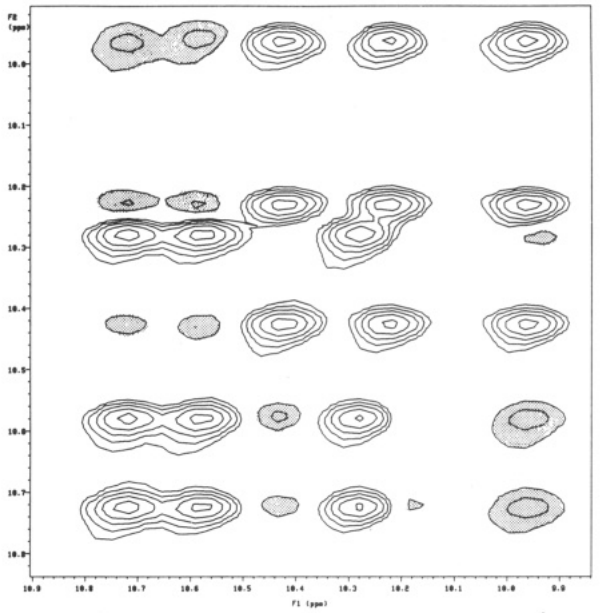


Figure 8. ROESY spectrum (phenolic protons) of **2** in $CDCl_3$ at 223 K. **Exchange peaks have the same sign as the diagonal.** Cross-relaxation peaks have sign opposite to the diagonal and appear shadowed.

according to the ring substitution. Cross-relaxation cross-peaks (with sign opposite to the diagonal) can be observed between phenolic protons belonging to the two groups using mixing times of 200 or 500 ms. Unfortunately, at 223 K exchange is faster than cross-relaxation and it is impossible to differentiate close contacts between individual protons of different sets or between protons within a set.

The six AX systems corresponding to the methylene units overlap in pairs, and the high-field doublets of two of the pairs also overlap giving a total of five resolved sites between which cross-relaxation or exchange can be detected (Figure 7).[21] At short mixing times[22] different exchange processes can be distinguished: (i) the scrambling of the low-field and the high-field sites of each set independently of each other giving the cross-peaks labeled *a* in Figure 7 and (ii) the interconversion of the high-field and low-field protons of two of the sets (labeled *b*). Combinations of these processes explain the other cross-peaks. Notice the absence of cross-peaks between the high-field and the low-field protons of the third set (the one at the highest field). At long mixing times these sites also exchange probably through a three-step process (e.g., i + ii + i).

Our experimental results in $CDCl_3$ at 223 K and CD_2Cl_2 at 183 K provide evidence for at least three types of conformational exchange processes.

(a) Pseudorotation of the Macrocyclic Ring. This process exchanges the phenolic rings that are projecting outside between three possibilities (Scheme Ib). The corresponding three-site exchanges between protons of **2** are summarized in Scheme Ia. The three phenolic rings that have the same substitution are interconverted by this process, and also each methylene proton exchanges with

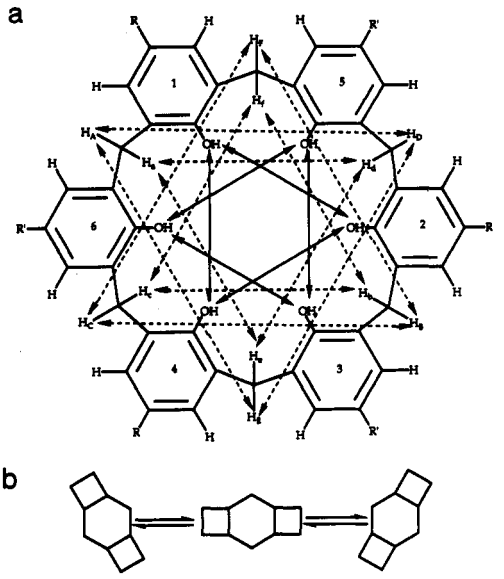
(19) Jaime, C.; de Mendoza, J.; Prados, P.; Nieto, P. M.; Sánchez, C. *J. Org. Chem.* **1991**, *56*, 3372–3376.

(20) Carramolino, M.; Nieto, P. M.; Prados, P.; de Mendoza, J. Unpublished results.

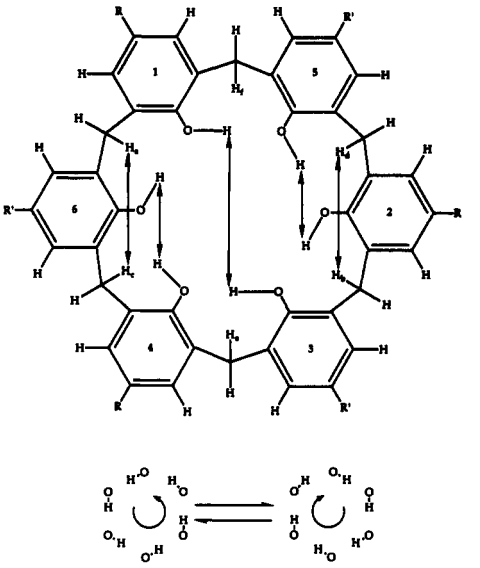
(21) Cross-peaks between protons in the methylene region are less well resolved in the NOESY and ROESY spectra than in the double quantum filtered COSY because of the in-phase nature of the former.

(22) At long mixing times (≥200 ms, data not shown) scrambling between all the sites is complete, explaining the singlet observed at room temperature for the methylene protons.

Scheme I



Scheme II



two others separated by two phenolic rings. This process does not exchange the methylene protons of high and low field. The experimental evidence coming from NOESY and ROESY experiments recorded in $CDCl_3$ at 223 K (see Figure 8 and Figure 7 peaks labeled *a*) is consistent with this mechanism. The rate constants measured from the 2D spectra (see Experimental Section) afford an energy barrier for this process of 12.2 ± 0.2 kcal mol^{-1}.

(b) Macrocyclic Ring Inversion. This is achieved by flipping of four phenol rings from an "up" to a "down" position and vice versa leaving the remaining two in the out position. This process exchanges the high- and low-field protons of the four methylene groups flanking the two "out" phenols rings. The experimental evidence for this process is also found in the NOESY and ROESY experiments recorded in $CDCl_3$ at 223 K. (See Figure 7 cross-peaks labeled *b*, k_{exch} = 1.4 s^{-1}, $\Delta G^{\ddagger}$ = 12.8 kcal mol^{-1}). The absence of a cross-peak between the high- and low-field protons of the remaining methylene groups (e/E and f/F) indicates that the two adjacent phenol rings move simultaneously "up" and "down" maintaining their syn arrangements. At long mixing times these protons can also exchange through a pathway involving a pseudorotation, followed by ring inversion and a second pseudorotation.

It is interesting to notice that at a given temperature (223 K) the conformational exchange by these two processes is much faster in CD_2Cl_2 than in $CDCl_3$. Computergraphics docking indicates that the cavity of calix[6]arenes has exactly the right size to accommodate a chloroform molecule, and the formation of an inclusion complex with the solvent could explain the observed effect. Work is in progress to investigate this possibility in different calix[6]arenes.

(c) Hydrogen Bond Reversal. This results in a change in the sense of rotation of the cyclic hydrogen bond array and interconverts enantiomeric conformations. This two-site process apparently exchanges protons related by the plane that bisects the two phenolic rings in "out" position (Scheme II). If the hydrogen bond reversal is rapid enough this plane would become a plane of symmetry. The exchange cross-peaks observed at 183 K in CD_2Cl_2 in the fully protonated sample can be explained by this mechanism and provide a rate constant of 1.0 s^{-1} ($\Delta G^{\ddagger}$ = 10.6 kcal mol^{-1}).

Surprisingly enough there is no evidence for this process in the NOESY and ROESY experiments performed at 223 K in $CDCl_3$. There are two conceivable mechanisms through which this hydrogen bond reversal can occur. One involves breaking the six hydrogen bonds and reorientation of the hydroxy groups, the second involves hydrogen exchange between each hydrogen bond donor and acceptor in a concerted fashion along the cyclic array. The rapid exchange at this low temperature and the fact that it is subjected to a strong isotopic effect ($k_H/k_D \geq 5$)[23] suggest that this exchange may proceed through a proton-tunneling mechanism.

Conclusions

We have assigned completely the ^{1}H-NMR spectrum of **2**, and we have been able to determine that at low temperature in CD_2Cl_2 solution this flexible ring is frozen in a winged-cone conformation with a cyclic array of hydrogen bonds that causes its complete lack of symmetry. We have also been able to characterize the exchange processes that lead to a completely symmetrical spectrum at room temperature. We believe that this is the first example of the determination of the three-dimensional structure of a calix[6]arene in solution and also the first analysis of the topoisomerism of this family of compounds,[24] and the results should be applicable to other calix[6]arenes. In particular, our studies suggest that hexa-*tert*-butylcalix[6]arene has an analogous conformational behavior, and probably other calix[6]arenes with free hydroxy groups and large substituents in the upper rim follow the same pattern.

Experimental Section

NMR Spectroscopy. Spectra were run at 500 MHz (^{1}H) and 125 MHz (^{13}C) on a Varian VXR-500 at temperatures ranging from 295 to 223 K (in $CDCl_3$) or to 183 K (in CD_2Cl_2). Temperatures

(23) The exchange is not detectable in the 50% deuterated sample. The overall rate constants therefore had to be at least a factor of 32 lower than in the fully protonated sample, considering the detection limit of the experiment. Nevertheless, the presence of six hydrogen bonds and the fact that the sample is statistically deuterated has to be taken into account. To evaluate the isotopic effect per hydrogen bond the isotopomer distribution and the number of protons in each of the species present was considered and expected rates were calculated as a function of the primary isotopic effect. We found that values larger than 5 would give experimentally indistinguishable results.

(24) Very recently the conformational interconversion of different calix[4]arenes containing a polyether bridge between alternate rings has been studied by 2D–NMR: van Loon, J. D.; Groenen, L. C.; Wijmenga, S. S.; Verboom, W.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1991**, *113*, 2378–2384.

were checked using a sample of methanol. Chemical shift values are quoted in parts per million (ppm) downfield from tetramethylsilane (TMS) employed as internal reference. Sample concentrations were 10–14 mM. A partially deuterated sample of **2** was prepared by adding methanol-d_4 to a chloroform solution of **2**, and the degree of deuteration was determined by integration of the phenolic protons. Afterwards, the solvents were evaporated and the solid dissolved in pure dichloromethane-d_2.

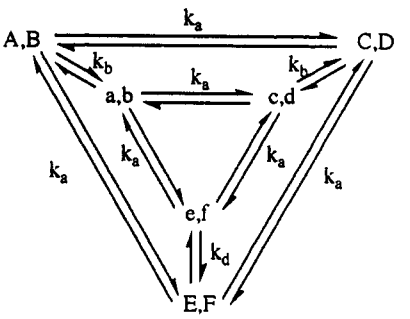
Homonuclear Correlation Spectroscopy. DQF-COSY[25] (double quantum filtered correlation spectroscopy), NOESY[26] (nuclear Overhauser spectroscopy), and ROESY[27] (rotating frame nuclear Overhauser spectroscopy) standard pulse sequences were used. All the experiments were collected in the phase-sensitive mode using 2D hypercomplex data (States–Haberkorn method[28]). A total of 2 × 256 increments of 2K spectra (eight scans) were collected and zero-filled to 1024 in F1 and 4096 in F2 for NOESY and ROESY spectra with mixing times ranging from 10 to 500 ms. For DQF-COSY spectra were collected with 4K points.

Heteronuclear Correlation Spectroscopy. HMQC[29] (heteronuclear multiple quantum correlation) and HMBC (heteronuclear multiple bond correlation) standard pulse sequences were used. All the experiments were collected in the phase-sensitive mode using the States–Haberkorn method. The HMBC experiments were usually analyzed in absolute value although in some cases where the highest resolution was needed we examined spectra phased in selected regions. In order to increase the digital resolution in F1 the spectral width of the carbon dimension of HMBC spectra was reduced to the regions of interest taking care that folded signals did not interfere.

The number of scans was typically 32 for HMBC (with t = 50–90 ms) and four for HMQC with experimental time of ~3 and ~1 h, respectively. A total of 2 × 256 increments of 2K spectra were collected and zero-filled to 1024.

Molecular Modeling. The three-dimensional structure of **2** was derived using the NMR data. Calculations were performed on a Silicon Graphics Personal IRIS 4D/35 using INSIGHTII and DISCOVER packages.[30] The CVFF[31] force field was used. A high-temperature dynamic simulation was initially run on an open analogue without any substituent in position 4 and using constraints for the hydroxy–hydroxy and the methylene–hydroxy short distances detected experimentally in the cyclic molecule. An artificial end to end distance was also used as a constrain and gradually decreased until a conformation was obtained that could be cyclized. Up to this point, the two methylene protons were represented by a single pseudoatom. After bonding the two extremes the still unsubstituted molecule was subjected to short dynamics at 500 K without electrostatic terms. At this stage, the stereospecific assignment of the methylene protons that gave the best fit between the model and the experimental data were applied and used for the final refinements. After adding the substituents, the structure was subjected to restrained dynamics at 500 K with the full electrostatic terms, annealed to 273 K, and minimized without constraints.

Calculation of Rate Constants. Approximate rate constants for the pseudorotation and ring inversion processes were obtained from integration of the peaks of a 200 ms ROESY experiment in $CDCl_3$ at 223 K. Data were processed using the method of Abel et al.,[32] neglecting cross-relaxation, assuming a single relaxation time for all the protons and considering a six-site exchange network.[33]



The rate constant for the hydrogen bond reversal process was obtained from a ROESY experiment in CD_2Cl_2 at 183 K using the equations for a two-site model

$$k_{exch} = (1/t_{2tm}) \ln [(1 + R)/(1 - R)] \qquad R = I_{ij}/I_{ii}$$

Acknowledgment. We acknowledge financial support from the Dirección General de Investigación Científica y Técnica (DGICYT grants PB91-283 and PB90-0212) and Comisión Interministerial de Ciencia y Tecnología (CICYT PB87-0107). M.A.M. holds a predoctoral grant from Farmhispania S.A. This work has been carried out using the facilities of the Serveis Científico-Tècnics de la Universitat de Barcelona.

(25) Bax, A. *J. Magn. Reson.* **1983**, *53*, 517–520.
(26) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546–4553.
(27) Bothner-By, A. A.; Stephens, R. L.; Lee, L.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813.
(28) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286–292.
(29) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285–4294.
(30) Commercially available from Biosym Technologies Inc.
(31) (a) Ermer, O.; Lifson, S. *J. Am. Chem. Soc.* **1973**, *95*, 4121–4132. (b) Hagler, A. T.; Lifson, S. *J. Am. Chem. Soc.* **1974**, *96*, 5319–5327. (c) Hagler, A. T.; Dauber, P.; Lifson, S. *J. Am. Chem. Soc.* **1979**, *101*, 5131–5141.
(32) Abel, E. W.; Coston, T. P. J.; Orrell, K. G.; Sik, V.; Stephenson, D. *J. Magn. Reson.* **1986**, *70*, 34–53.
(33) Labels are the same as in Scheme I.