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Isotopic Perturbation of the Conformational Equilibrium in Methylene-Functionalized Calixarenes

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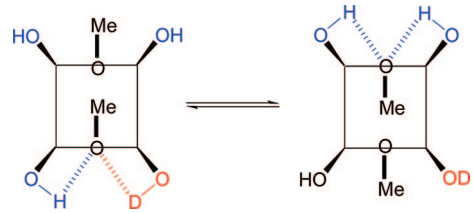

The 400 MHz ^{1}H NMR spectrum of the tetramethoxycalixarene **2** (possessing hydroxyl groups at the bridges) in commercial acetone-d_6 displays five signals (an isotopic multiplet) for a pair of methoxy groups. Inspection of the X-ray structures of **2** and its isomer **3** indicates that in the adopted 1,3-alternate conformation, the methoxy groups intramolecularly hydrogen bonded to neighboring OH groups are oriented "in" (pointing toward the cavity). Upon dissolution of **2** in acetone-d_6, none, some, or all of the OH protons exchange with the deuterium atoms present in the residual water of the solvent. Several species (mutually relating as isotopomers and isotopologues) differing in the number and positions of the deuterated hydroxyl groups are possible for **2**. In three of these species, the "in"−"out"/"out"−"in" conformational equilibrium of a pair of methoxy groups is nondegenerate. The four external lines of the apparent multiplet are ascribed to a single- and double-isotopic perturbation of the "in"−"out" conformational equilibrium of a pair of methoxy groups. On the basis of the assignment of the signals to the individual species and their statistical distribution, the intensities of the components of the isotopic multiplet obtained at different isotopic enrichments of the hydroxyl groups could be simulated. A sample of **2** 55% deuterated at the hydroxyl groups in $CDCl_3$ displayed an isotopic multiplet consisting of nine signals. The isotopic multiplet observed for the OH groups of **2** in acetone-d_6 was simulated at different deuteration enrichments.

Introduction

The calix[4]arenes are useful synthetic platforms for the construction of supramolecular hosts. In most cases, functionalization of the calix scaffold with specific functional groups is desirable. Although a multitude of synthetic methods are available for the modification of the aryl groups of the calixarenes,[1] only few have been reported allowing the functionalization of their methylene groups.[2] Calixarenes incorporating carbonyl groups at the bridges (ketocalixarenes) may serve as starting materials for the preparation of such systems.[3] With that goal in mind, we recently examined the reaction of ketocalixarene **1** with excess PhLi.[4] The reaction afforded a mixture of all possible stereoisomers of the tetra-addition product (*rccc*, *rcct*, *rtct*, and *rctt*),[5] but upon crystallization from chloroform/acetone the *rccc* form (i.e., *all-cis*, **2**) could be

(1) For reviews on calixarenes, see: (a) *Calixarenes, a Versatile Class of Macrocyclic Compounds*; Vicens, J., Böhmer, V., Eds.; Kluwer: Dordrecht, 1991. (b) Böhmer, V. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 713. (c) Gutsche, C. D. *Aldrichim. Acta* **1995**, *28*, 1. (d) Pochini, A.; Ungaro, R. In *Comprehensive Supramolecular Chemistry*; Vögtle, F., Vol. Ed.; Pergamon Press: Oxford, UK, 1996; Vol. 2, p 103. (e) Gutsche, C. D. *Calixarenes Revisited*; Royal Society of Chemistry: Cambridge, 1998. (f) *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J., Eds.; Kluwer: Dordrecht, 2001. (g) Böhmer, V. In *The Chemistry of Phenols*; Rappoport, Z., Ed.; Wiley: Chichester, 2003; Chapter 19.

(2) See, for example: (a) Tabatai, M.; Vogt, W.; Böhmer, V. *Tetrahedron Lett.* **1990**, *31*, 3295. (b) Sartori, G.; Maggi, R.; Bigi, F.; Arduini, A.; Pastorio, A.; Porta, C. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1657. (c) Klenke, B.; Näther, C.; Friedrichsen, W. *Tetrahedron Lett.* **1998**, *39*, 8967. (d) Middel, O.; Greff, Z.; Taylor, N. J.; Verboom, W.; Reinhoudt, D. N.; Snieckus, V. *J. Org. Chem.* **2000**, *65*, 667. (e) Agbaria, K.; Biali, S. E. *J. Am. Chem. Soc.* **2001**, *123*, 12495. (f) Scully, P. A.; Hamilton, T. M.; Bennett, J. L. *Org. Lett.* **2001**, *3*, 2741. (g) Kumar, S.; Chawla, H. M.; Varadarajan, R. *Tetrahedron Lett.* **2002**, *43*, 7073. (h) Columbus, I.; Biali, S. E. *J. Org. Chem.* **2008**, *73*, 2598.

(3) (a) Görmar, G.; Seiffarth, K.; Schultz, M.; Zimmerman, J.; Flämig, G. *Makromol. Chem.* **1990**, *191*, 81. See also: (b) Seri, N.; Simaan, S.; Botoshansky, M.; Kaftory, M.; Biali, S. E. *J. Org. Chem.* **2003**, *68*, 7140.

(4) Kuno, L.; Seri, N.; Biali, S. E. *Org. Lett.* **2007**, *9*, 1577.

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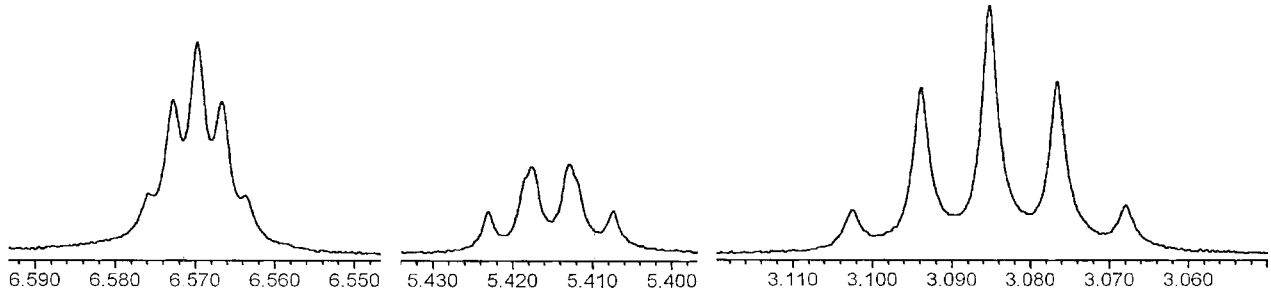
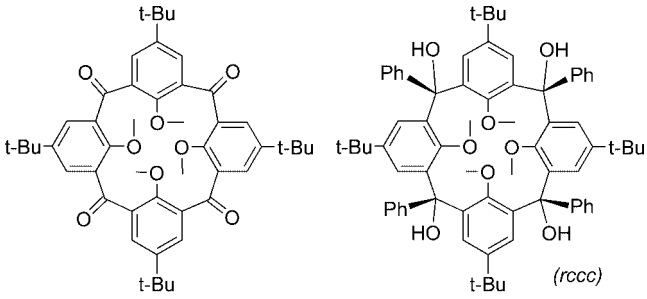


FIGURE 1. 400 MHz ^{1}H NMR spectrum of selected regions (*tert*-butylated aromatic ring at 6.57 ppm, OH and low-field methoxy group signal) of **2** in acetone-d_6 containing ca. 50% D in the residual water after dissolution of the sample.

isolated.[4] X-ray crystallography indicated that the calix macrocycle adopts the 1,3-alternate conformation.[6,7]



The tetrahydroxytetraphenylcalix[4]arene derivative **2** displayed in the ^{1}H NMR spectrum in $CDCl_3$ (400 MHz, rt) two methoxy singlets at 3.01 and 2.47 ppm, in agreement with a frozen (on the NMR time scale) 1,3-alternate conformation. Unexpectedly, when the NMR of **2** was measured in acetone-d_6, the low-field methoxy signal resembled the familiar shape of a quintet (Figure 1). The present study was conducted to investigate this phenomenon.

Results and Discussion

Isotopic Multiplet. The separation (in Hz) between the proton signals forming the apparent quintet (3.4 Hz at 400 MHz, 8.5 ppb) of the low-field methoxy group signal of **2** become proportionally smaller when measured with a 300 MHz NMR instrument (2.6 Hz, 8.5 ppb), indicating that the apparent splitting observed is not due to coupling interactions. The apparent quintet shape can be therefore ascribed to the presence of several nearly equally spaced singlets that possess an intensity pattern resembling a quintet. The apparent splitting disappeared when D_2O was added to the solution. Since the residual water of the commercial sample of acetone-d_6 used was partially deuterated, and the splitting of the methoxy signal disappeared when the sample in acetone-d_6 was shaken with D_2O, it can be concluded that the pattern observed (which from now on will be referred as the isotopic multiplet) is connected to the presence of species in which none, part, or all of the OH protons have been exchanged by deuterons. Splitting was also observed for the OH signal and for one of the two signals of the aromatic protons of the *tert*-butylated rings (resonating at δ 6.57 ppm, Figure 1). It seems unlikely that the latter selective splitting (a long-range effect caused by the deuteration) is due to an intrinsic deuterium isotope effect on the chemical shifts. In addition, the symmetry of the isotopic multiplet of the methoxy signals was not affected by a change in the isotopic enrichment (see below). On this basis, we conclude that, most likely, the appearance of an isotopic multiplet for a pair of methoxy groups of **2** is the result of an isotopic perturbation of an otherwise degenerate equilibrium[8−10] in some of the species present in the partially deuterated sample.[11,12] If so, which degenerate equilibrium of **2** is perturbed?

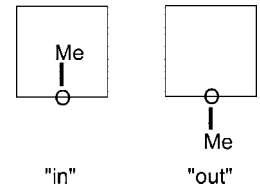


FIGURE 2. Schematic representation of the "in" and "out" orientations of a given methoxy group in a methoxy calixarene. The square represents a top view of the calix[4]rene skeleton. The corners and edges of the square represent the methylene groups and aryl rings, respectively.

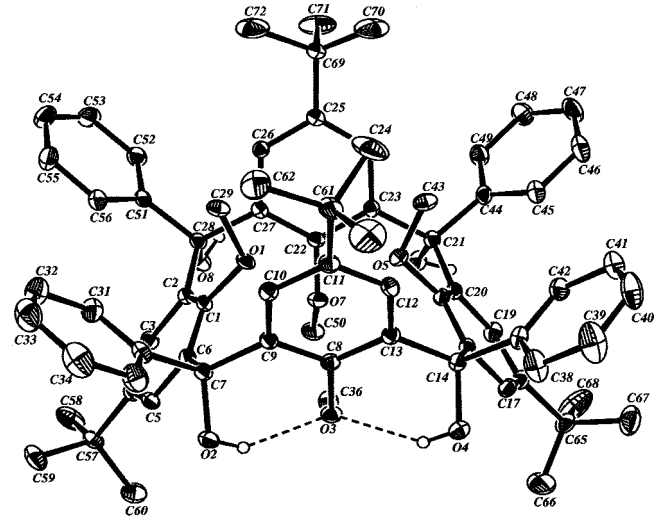


FIGURE 3. X-ray structure of **2**.[4]

Conformation of the Methoxy Groups of 2. Assuming that in their preferred conformation the methoxy groups are oriented

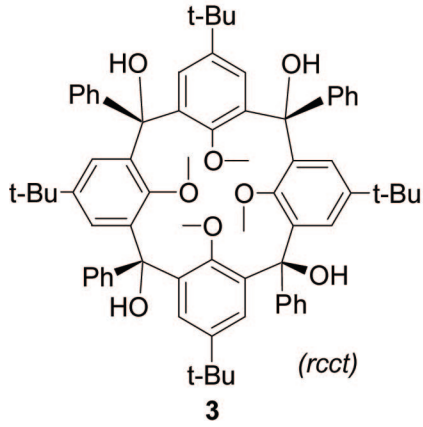
(5) Four isomers are possible for a calix[4]arene derivative with the four bridges identically substituted (see ref 4). These forms are configurational isomers and not conformers since their mutual interconversion requires cleavage of bonds.

(6) The conformation of calix[4]arenes is usually discussed in terms of four basic forms (cone, partial cone, 1,3-alternate, and 1,2-alternate, ref 1) arising form the different possible "up" or "down" arrangements of the aryl rings. Rotation through the annulus of the rings mutually interconverts these forms.

(7) A calix[4]arene of unknown stereochemistry monofunctionalized at four bridges with OH groups was prepared by Görmar and coworkers via $LiAlH_4$ reduction of a ketocalixarene. See ref 3a.

(8) Anet, F. A. L.; Basus, V. J.; Hewett, A. P.W.; Saunders, M. *J. Am. Chem. Soc.* **1980**, *102*, 3945.

(9) For a review, see: (a) Siehl, H.-U. *Adv. Phys. Org. Chem.* **1987**, *23*, 63.



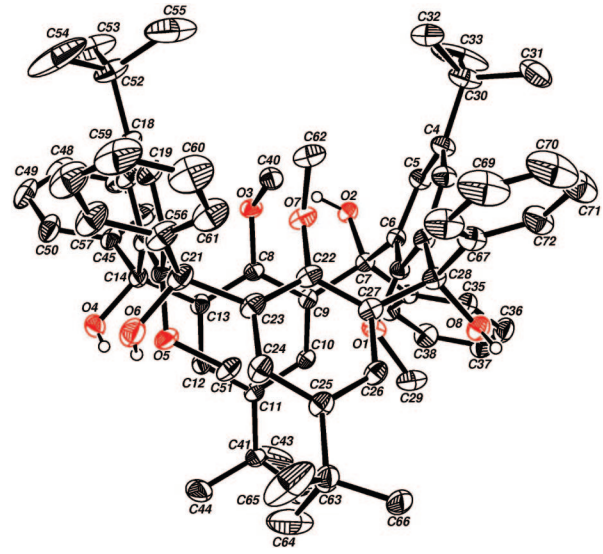
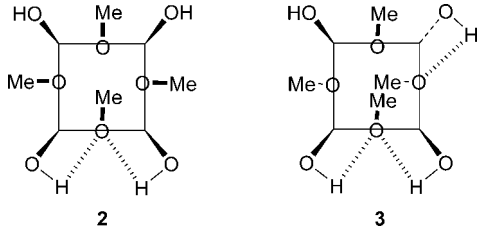
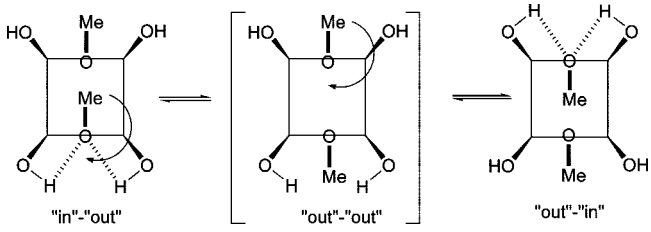


FIGURE 4. X-ray structure of **3**.

SCHEME 1. Hydrogen-Bonding Patterns in 2 and 3



SCHEME 2. "Flip Flop" Motion of Two Methoxy Groups



perpendicular to the aryl rings to which they are attached, two possible conformations ("in" and "out") are possible (Figure 2). In calixarene tetramethyl ethers, "out" arrangements of the methoxy groups are usually observed.[13]

Calixarene **2** possess an *rccc* disposition of hydroxy (and phenyl) substituents and in the crystal adopts a 1,3-alternate conformation (Figure 3).[4] For identification purposes, pairs of

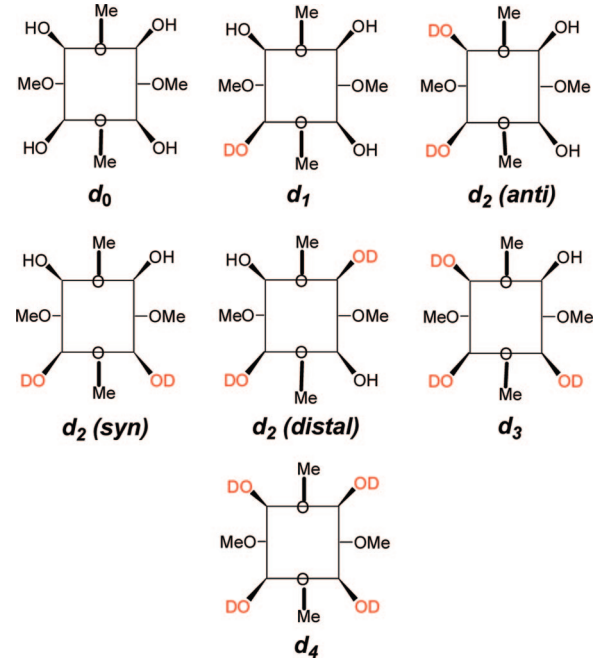


FIGURE 5. Schematic representation of the seven different species resulting from H/D exchange of the hydroxyl groups of **2**. For simplicity, all the methoxy groups are drawn in an "out" conformation.

hydroxyl groups flanking a given aryl ring will be referred as "syn" (O2-H/O4-H and O6-H/O8-H) and "anti" (O2-H/O8-H and O4-H/O6-H). Notably, the two methoxy groups located

(10) For selected applications of the isotopic perturbation method, see, for example: (a) Saunders, M.; Kates, M. R. *J. Am. Chem. Soc.* **1977**, *99*, 8071. (b) Faller, J. W.; Murray, H. H.; Saunders, M. *J. Am. Chem. Soc.* **1980**, *102*, 2306. (c) Ahlberg, P.; Engdahl, C.; Jonsall, G. *J. Am. Chem. Soc.* **1981**, *103*, 1583. (d) Anet, F. A. L.; Kopelevich, M. *J. Am. Chem. Soc.* **1986**, *108*, 2109. (e) Saunders, M.; Jarret, R. M.; Pramanik, P. *J. Am. Chem. Soc.* **1987**, *109*, 3735. (f) Hansen, P. E. *Acta Chem. Scand. B* **1988**, *42*, 423. (g) Perrin, C. L.; Thoburn, J. D. *J. Am. Chem. Soc.* **1989**, *111*, 8010. (h) Anet, F. A. L.; O'Leary, D. J.; Williams, P. G. *J. Chem. Soc., Chem. Commun.* **1990**, 1427. (i) Mazzini, S.; Merlini, L.; Mondelli, R.; Nasini, G.; Ragg, E.; Scalioni, L. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2013. (j) Ahlberg, P.; Davidsson, O.; Lowendahl, M.; Hilmersson, G.; Karlsson, A.; Hakansson, M. *J. Am. Chem. Soc.* **1997**, *119*, 1745. (k) Quast, H.; Seefelder, M.; Becker, C.; Heubes, M.; Peters, E.-M.; Peters, K. *Eur. J. Org. Chem.* **1999**, 2763. (l) Perrin, C. L.; Kim, Y.-J. *Inorg. Chem.* **2000**, *39*, 3902. (m) Perrin, C. L.; Ohta, B. K. *J. Am. Chem. Soc.* **2001**, *123*, 6520. (n) Siehl, H.-U.; Vrcek, V.; Kronja, O. *J. Chem. Soc., Perkin Trans. 2* **2002**, 106.

(11) For examples of isotopic multiplets in polyols, see: (a) Reuben, J. *J. Am. Chem. Soc.* **1983**, *105*, 3711. (b) Reuben, J. *J. Am. Chem. Soc.* **1984**, *106*, 6180. (c) Reuben, J. *J. Am. Chem. Soc.* **1985**, *107*, 1756.

(12) See also: (a) Christofides, J. C.; Davies, D. B. *J. Am. Chem. Soc.* **1983**, *105*, 5099. (b) Christofides, J. C.; Davies, D. B. *J. Chem. Soc., Perkin Trans. 2* **1987**, 97. (c) Craig, B. N.; Janssen, M. U.; Wickersham, B. M.; Rabb, D. M.; Chang, P. S.; O'Leary, D. J. *J. Org. Chem.* **1996**, *61*, 9610. (d) Lewis, B. E.; Schramm, V. L. *J. Am. Chem. Soc.* **2001**, *123*, 1327. (e) Vasquez, T. E., Jr.; Bergset, J. M.; Fierman, M. B.; Nelson, A.; Roth, J.; Khan, S. I.; O'Leary, D. J. *J. Am. Chem. Soc.* **2002**, *124*, 2931. (f) Anderson, C. E.; Britt, D. K.; Sangji, S.; O'Leary, D. J.; Anderson, C. D.; Rychnovsky, S. D. *Org. Lett.* **2005**, *7*, 5721. (g) Anderson, C. E.; Pickrell, A. J.; Sperry, S. L.; Vasquez, T. E., Jr.; Custer, T. G.; Fierman, M. B.; Lazar, D. C.; Brown, Z. W.; Iskenderian, W. S.; Hickstein, D. D.; O'Leary, D. J. *Heterocycles* **2007**, *72*, 469.

(13) Statistical analysis of the crystal structure data of the methoxyphenyl group in different environments has shown that the perpendicular conformation of the methoxy group is preferred if the two ortho positions are substituted. See: Hummel, W.; Huml, K.; Bürgi, H.-B. *Helv. Chim. Acta* **1988**, *71*, 1291.

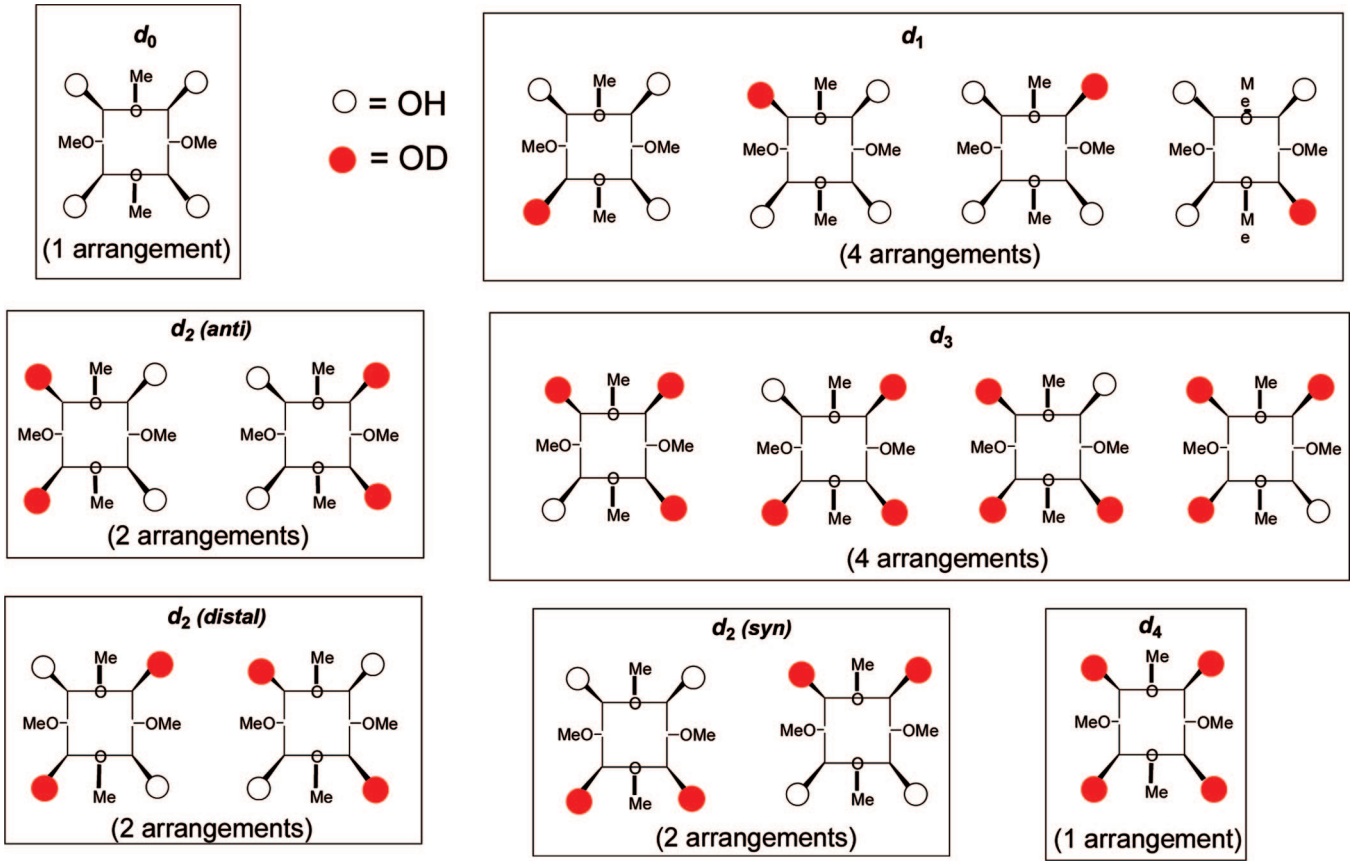


FIGURE 6. Number of possible arrangements for the OH (white circles) or OD (red circles) groups. The relative number of arrangements represents the statistical populations of the seven forms in a sample 50% deuterated.

between the "syn" pairs of OH groups (O3−C36 and O7−C50) are oriented in an "in"−"out" fashion, with the "in" methoxy group (O3−C36) hydrogen bonded (the methoxy serves as a bifurcated acceptor) to the two neighboring OH groups. The second pair of methoxy groups (O1−C29 and O5−C43), which is oriented "out", is located in the opposite hemisphere to the four OH groups and therefore cannot be involved in intramolecular hydrogen bonds. It seems likely that in the 1,3-alternate conformation of the macrocycle the "in" orientation of a methoxy group is better suited than the "out" orientation for the formation of hydrogen bonds with the hydroxyl groups at the bridges. In the "in" conformation, the lone pairs on the oxygen atom of the methoxy groups are oriented toward the exterior of the molecule, in steric proximity to the OH proton. An "in" orientation of both methoxy groups O3−C36 and O7−C50, although enabling two pairs of bifurcated hydrogen bonds, is more likely to be destabilized by steric interactions between the pair of methoxy groups in close steric proximity, and therefore, such arrangement is not preferred.

Crystal Structure of 3. Calixarene **3** was previously obtained as one of the products of the addition of PhLi to ketocalixarene **1**.[4] Compound **3** differs from **2** in the configuration of the stereocenters at the carbon bridges (*rcct* vs *rccc*, respectively), and therefore, the two compounds differ in the geometric disposition of OH groups. The crystal structure of **3** was determined to further support the hypothesis that in the 1,3-alternate conformation of the macrocycle, intramolecular hydrogen bonding of a methoxy group with a neighboring OH group requires an "in" orientation of the methoxy. According to the X-ray structure, the calix macrocycle of **3** also adopts an 1,3-alternate conformation (Figure 4). Three hydroxyl groups are located in one hemisphere of the molecule, with a single OH group (trans to the rest) located in the opposite hemisphere. Two methoxy groups of **3** are intramolecularly hydrogen bonded, one (O5−C51) in bifurcated fashion to a pair of neighboring OH groups and one (O3−C40) in a simple fashion. As observed for **2**, both hydrogen-bonded methoxy groups are oriented "in". Since the pair of methoxy groups oriented "in" are located at different hemispheres, this arrangement is not destabilized by steric effects. A schematic representation of the hydrogen bonding pattern and conformation of the methoxy groups of **2** and **3** is shown in Scheme 1. From inspection of the crystal structures of **2** and **3**, it is readily apparent that in the 1,3-alternate conformation only hydroxy groups located at the same hemisphere as a neighboring methoxy group can form a hydrogen bond with it, and this requires an "in" orientation of that methoxy group.

Solution Conformation of 2. The ^{1}H NMR spectrum of **2** in $CDCl_3$ displays two signals each for the methoxy, *t*-Bu and *tert*-butylated aromatic groups. This pattern of signals is only compatible with the *rccc* isomer adopting a frozen 1,3-alternate conformation on the NMR time scale, the two methoxy signals ascribed to the two types of methoxy groups (the methoxy groups between pairs of syn and anti OH groups are symmetry nonequivalent). Raising the temperature of a sample of **2** in 1,1,2,2-tetrachloroethane-d_2 up to 408 K did not result in any appreciable broadening of the methoxy signals, but new signals slowly appeared corresponding to the *rcct* form, indicating that at relatively high temperatures **2** undergoes thermal isomeriza-

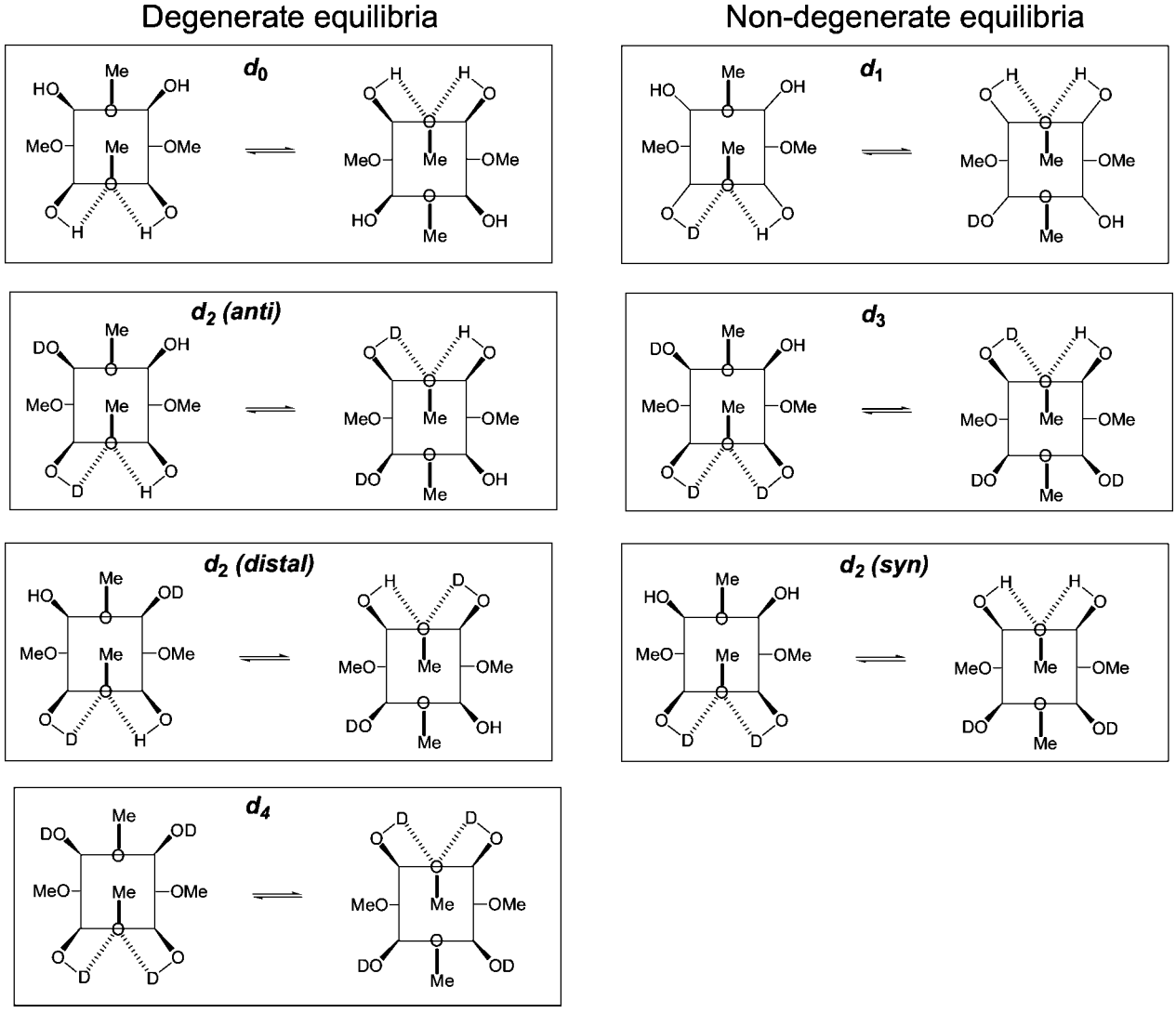


FIGURE 7. Degenerate and nondegenerate "in"−"out"/"out"−"in" equilibrium.

tion.[14] In the following discussion, we will assume that the conformation of **2** found in the crystal (including the two intramolecular hydrogen bonds) is the one preferred in solution: i.e., a 1,3-alternate conformation of the macrocycle with a "in"−"out" arrangement of a pair of methoxy groups. However, since in the ^{1}H NMR spectrum in $CDCl_3$ at room temperature these two groups display a single signal, it seems reasonable to assume that the "in"−"out" conformation is in rapid exchange (on the NMR time scale) with the "out"−"in" form (Scheme 2). This process (a "flip flop" motion) involves stepwise 180° rotation around the Ar−OMe bonds of two methoxy groups (most likely via an "out"−"out" intermediate) and transforms the "out" methoxy group into an "in" group and vice versa. The low-field methoxy signal appearing as an isotopic multiplet is assigned to the methoxy groups rapidly rotating between the "in"−"out" and "out"−"in" orientations, while the high-field methoxy signal not showing any splitting is assigned to the methoxy groups pointing "out" (O1−C29 and O5−C43 in Figure 3).

Isotopomers and Isotopologues of 2. Upon dissolving a sample of **2** in acetone-d_6 containing deuterons in the residual water, H/D exchange takes place resulting in the formation of several species. In our analysis, we will assume that H/D exchange between the forms is slow on the NMR time scale.[15] The seven possible forms (which pairwise relate as either isotopomers or isotopologues)[16] are displayed in Figure 5. The ***d*$_1$**, ***d*$_3$**, and ***d*$_2$*(distal)*** forms are chiral and exist as enantiomeric pairs, while the ***d*$_0$**, ***d*$_4$**, ***d*$_2$*(syn)***, and ***d*$_2$*(anti)*** species are achiral.

Assuming D/H fractionation factors close to unity,[17] the relative populations of a sample 50% deuterated can be readily estimated on the basis of statistical considerations (Figure 6). On this basis, it can be concluded that the relative populations of the ***d*$_1$** and ***d*$_2$*(distal)*** forms should be four and two times larger than the population of the ***d*$_4$** form, respectively. The relative populations of the seven forms in a sample possessing a known deuteration different from 50% can be readily calculated. If "X_H" and "X_D" represent the fractions of protium and deuterium present in the sample ($X_H + X_D = 1$) then the

(14) This epimerization process most likely involves cleavage of the C−OH bonds and is probably catalyzed by acidic impurities in the solvent.

(15) It has been shown by Reuben for partially deuterated polyols (ref 11), that the chemical exchange between proton and deuteron in the hydroxy groups is slow on the NMR timescale.

(16) Two species are isotopologues if they differ only in the number of isotopic substitutions (i.e., the isotopic composition). Isotopomers refer to isomers differing only in the position of the isotopic substitution in the skeleton. See: Muller, P. *Pure Appl. Chem.* **1994**, *66*, 1077.

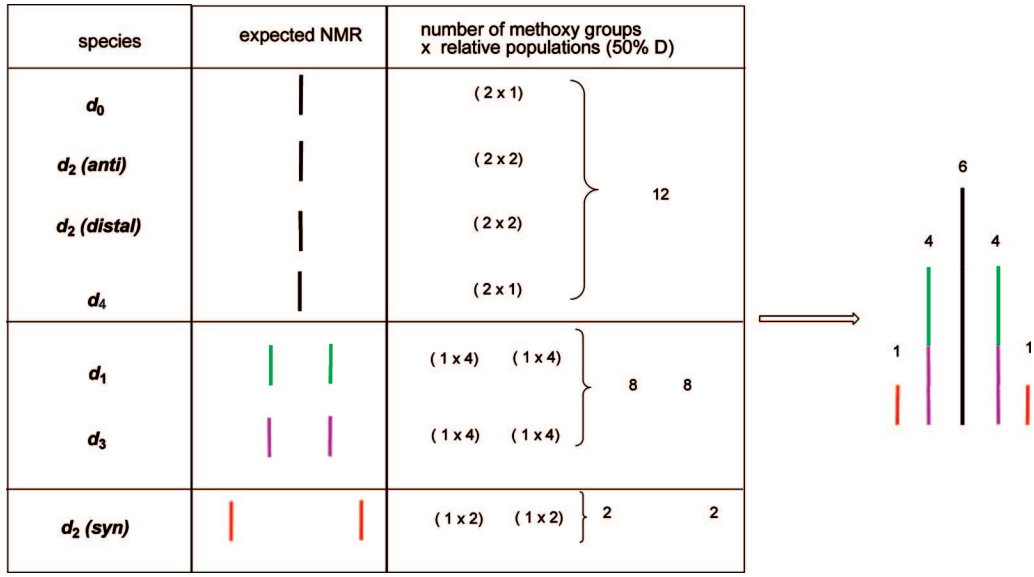


FIGURE 8. Assembling the isotopic multiplet of the low-field methoxy group from its individual components. The numbers on the five signals of the isotopic multiplet resembling a quintet represent the expected relative integrations of the signals.

statistical relative populations of the seven forms can be calculated as shown in eqs 1−5.

$$\text{population of } \boldsymbol{d_0}{:}\, 1 \times X_H^{\,4} \tag{1}$$

$$\text{population of } \boldsymbol{d_2(anti)} = \text{population of } \boldsymbol{d_2(distal)} =$$

$$\text{population of } \boldsymbol{d_2(syn)}{:}\; 2 \times X_H^{\,2} \times X_D^{\,2} \tag{2}$$

$$\text{population of } \boldsymbol{d_4}{:}\, 1 \times X_D^{\,4} \tag{3}$$

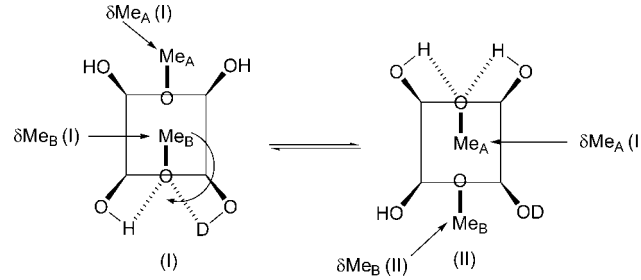
$$\text{population of } \boldsymbol{d_1}{:}\, 4 \times X_H^{\,3} \times X_D \tag{4}$$

$$\text{population of } \boldsymbol{d_3}{:}\, 4 \times X_H \times X_D^{\,3} \tag{5}$$

Isotopic Perturbation of the Conformational Equilibrium. In the nonlabeled compound, the "in"−"out" and "out"−"in" conformations (Scheme 2) are degenerate. Labeling of the OH groups may lift the degeneracy of this conformational equilibrium. However, not all labeling patterns will result in two nondegenerate conformations. As shown in Figure 7, only in the $\boldsymbol{d_1}$, $\boldsymbol{d_3}$, and $\boldsymbol{d_2(syn)}$ forms are the two rapidly interconverting conformations nondegenerate. For example, whereas in the "in"−"out" form of $\boldsymbol{d_1}$ the methoxy group oriented "in" is hydrogen bonded to OH and OD groups, in the "out"−"in" form the methoxy group oriented "in" is hydrogen bonded to two OH groups (Scheme 3). The difference in strength between hydrogen bonds involving OH and OD groups should remove the degeneracy of the two conformers and result in a non-zero energy gap between the two forms.

The perturbation of the degenerate conformational equilibrium should result in anisochronicity of the methoxy groups O−C36 and O−C50 of the $\boldsymbol{d_1}$, $\boldsymbol{d_3}$, and $\boldsymbol{d_2(syn)}$ forms. For example, since the "in"−"out" and "out"−"in" conformations of $\boldsymbol{d_1}$ are nondegenerate, the molar fraction of the conformation I (X(I), Scheme 3) should be slightly different than the molar fraction of the conformation II (X(II)). Disregarding isotope effects on the chemical shifts, the experimentally observed chemical shifts of the methoxy groups Me_A and Me_B (δ Me_A(obs) and δ Me_B(obs)) should be the weighted averages of their chemical shifts in the conformations I ($\delta Me_A(I)$ and ($\delta Me_B(I)$) and II ($\delta Me_A(II)$ and ($\delta Me_B(II)$) (Scheme 3, eqs 6 and 7):

SCHEME 3. Chemical Shifts in the Two Non-degenerate Conformations of d_1



$$\delta Me_A(\text{obs}) = X(I)\delta Me_A(I) + X(II)\delta Me_A(II) \tag{6}$$

$$\delta Me_B(\text{obs}) = X(I)\delta Me_B(I) + X(II)\delta Me_B(II) \tag{7}$$

To a first approximation, the perturbations of the conformational equilibria of $\boldsymbol{d_1}$ and $\boldsymbol{d_3}$ are expected to be similar, and therefore, the chemical shift difference between the two methoxy groups ($\Delta\delta = \delta Me_A(\text{obs}) - \delta Me_B(\text{obs})$, the "splitting" of the pair of signals) is expected to be nearly identical. In the case of the $\boldsymbol{d_2(syn)}$ form, the perturbation of the degeneracy is expected to be twice as large as the perturbation of either the $\boldsymbol{d_1}$ or $\boldsymbol{d_3}$ forms, since the two interconverting forms differ in the isotopic nature of two hydrogen bonds, and therefore the $\Delta\delta$ value for the pair of methoxy groups, is expected to be twice as large. The central line of the isotopic multiplet of the methoxy groups O3−C36 and O7−C50 is assigned to the accidentally isochronous signals of the $\boldsymbol{d_0}$, $\boldsymbol{d_2(anti)}$, $\boldsymbol{d_2(distal)}$, and $\boldsymbol{d_4}$ forms for which the "in"−"out" and "out"−"in" forms are degenerate, while the lines flanking it are assigned to the $\boldsymbol{d_1}$ and $\boldsymbol{d_3}$ forms. Finally, the two external lines of the isotopic multiplet are assigned to the $\boldsymbol{d_2(syn)}$ form.

The isotopic perturbation of the equilibrium should also be manifested in the NMR signals of other groups on the macrocycle. No splitting was observed for the phenyl rings at the bridges, since these signals are broad at room temperature due to restricted rotation of the phenyl rings.[4] The aromatic

(17) For a study on hydrogen isotope fractionation factors, see, for example: Guo, H. X.; Kresge, A. J. *J. Chem. Soc., Perkin. Trans. 2* **1997**, 295.

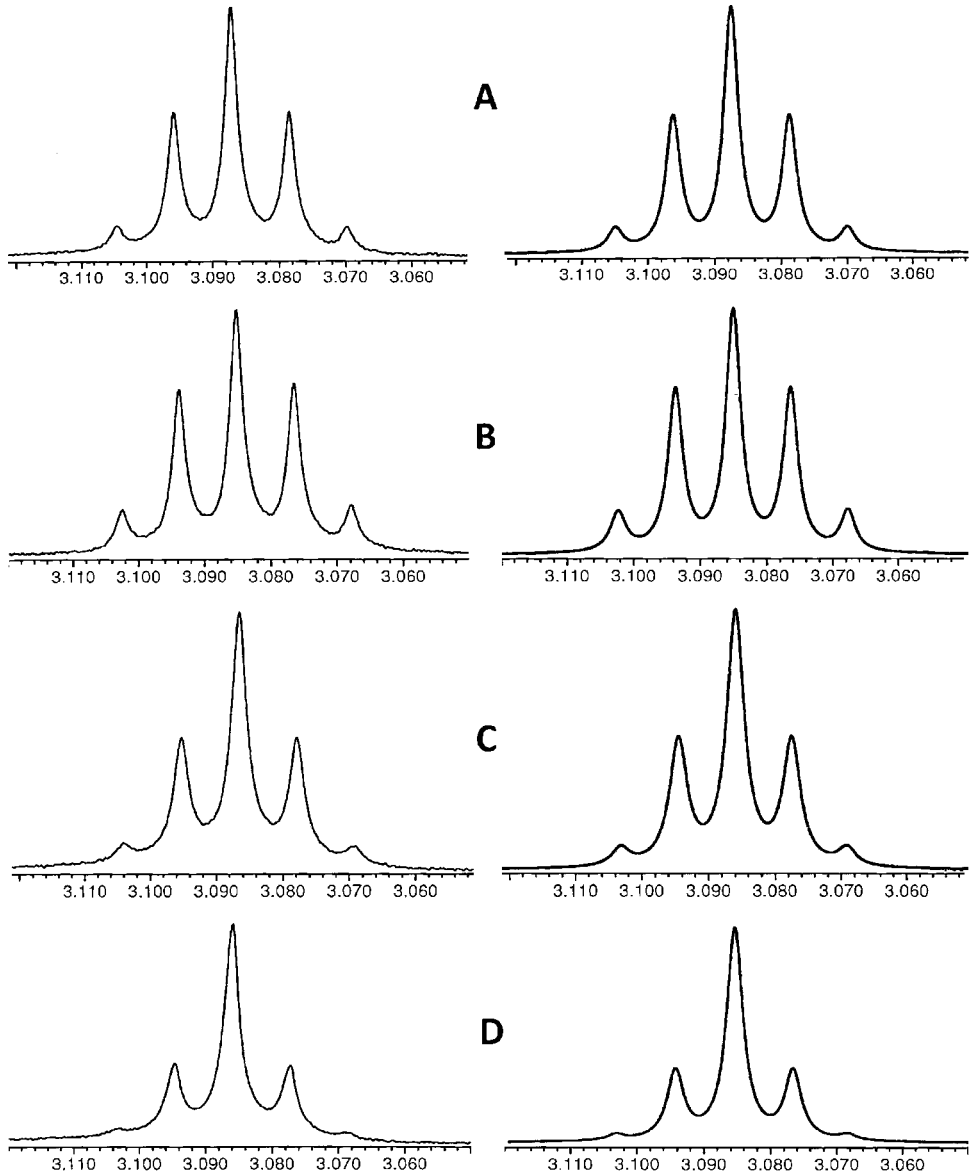


FIGURE 9. Experimental (left) and simulated (right) ^{1}H NMR spectrum (400 MHz) of the low-field methoxy signal of **2** in acetone-d_6 (A): 28%, (B), 50%, (C) 76%, and (D) 85% deuterated at the hydroxyl groups.

signal at δ 6.57 ppm which is split (Figure 1) is assigned to the aromatic protons at C5, C7, C3, and C19, which due to their proximity to the "in" methoxy group are most likely affected by the perturbation of the equilibrium.

Assembling the Isotopic Multiplet from the Individual Components. Based on the analysis above, the isotopic multiplet of the low field methoxy group can be assembled taking into account the individual contributions to the intensity of the five signals, which is a function of the relative population of each species, and the relative integration of the methoxy group signal(s) (i.e., whether the contribution of a given species is an average signal representing two methoxy groups or two individual signals each corresponding to one methoxy group). Assuming isochrony of the single signals of the d_0, d_2(*anti*), d_2(*distal*), and d_4 forms on one hand and the two signals of the d_1 and d_3 on the other hand, the spectrum can be modeled as shown schematically in Figure 8 for a sample 50% D labeled at the OH groups. The expected integration ratio of the five signals of the isotopic multiplets are 1:4:6:4:1.

Variation of the Isotopic Enrichment. On the basis on the proposed assignment of the signals to the individual species, the relative intensities of the signals of the isotopic multiplet for different percentages of deuteration can be simulated.[18] The relative populations of the seven forms and the relative intensities of the five transitions of the isotopic multiplet were calculated for each isotopic composition and the overall spectrum simulated using the gNMR program. The experimental and simulated spectra are shown in Figure 9. The agreement between the two sets of spectra is excellent, supporting the proposed assignment of the signals. Visual comparison of the spectra B and D in Figure 9 shows that the larger the departure from 50% deuteration, the smaller the two external transitions of the isotopic multiplet. This can be rationalized, since these transitions are assigned to the *2OD*(*syn*) form and its molar fraction decreases the larger the deviation from 50% deuteration.

(18) The percent of deuteration was determined from the ratio between the integration of the HOD signal and the sum of the total integration of the H_2O and HDO signals.

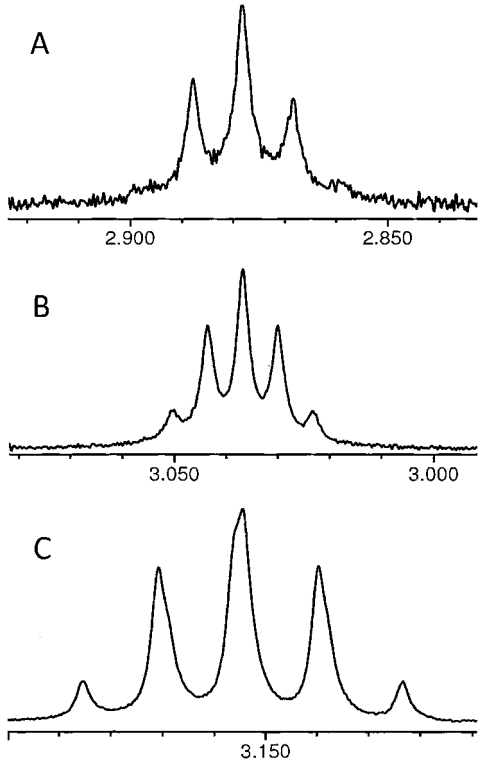


FIGURE 10. 400 MHz NMR spectrum of the low-field methoxy signal of **2** in different solvents: (A) DMSO-d_6, (B) THF-d_8, (C) C_6D_6.

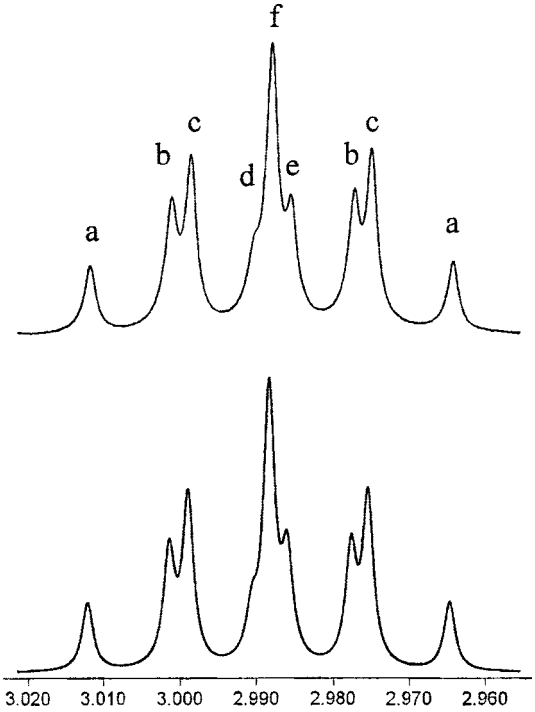


FIGURE 11. Experimental (top) and simulated spectra of the low-field methoxy signal of **2** 55% deuterated at the OH groups in $CDCl_3$.

The symmetric patterns observed at different isotopic enrichments support the hypothesis that the splitting of the signals is due to a single and double perturbation of the conformational equilibrium. If the transitions of the isotopic multiplet observed at 50% deuteration were due to an intrinsic isotope effect on the chemical shifts, the two external transitions (of lowest intensity) would correspond to the $\boldsymbol{d_0}$ and $\boldsymbol{d_4}$ forms (the two forms with the lowest statistical population). In such case, changing the deuterium enrichments should increase the population of one of the forms relative to the other, resulting in a nonsymmetric pattern. However, only symmetric patterns were observed, ruling out that the splittings observed are solely due to an intrinsic isotope effect.

Solvent Effects. In principle, if intermolecular hydrogen bonding of the solvent with **2** can successfully compete with the intramolecular hydrogen bonds, it could be expected that such interaction should decrease the perturbation of the degeneracy of the four species. Thus, under the assumption that the chemical shift difference between an "exo" and "endo" methoxy group is not affected by the nature of the solvent, a decrease in the separation between the signals in the isotopic multiplet (as compared to acetone-d_6, 8.5 ppb) should be expected in strong hydrogen-bond accepting solvents. To determine the influence of the nature of the solvent on the splitting of the isotopic multiplet, the 400 MHz ^{1}H NMR spectrum of **2** was determined in DMSO-d_6, THF-d_8, and C_6D_6 to which if needed were added small amounts of D_2O. In all solvents (including DMSO-d_6), the high-field methoxy signal appeared as a multiplet indicating that even in good hydrogen bond-donating solvents, a perturbation of the conformational equilibrium is observed. The signal's separation (DMSO-d_6: 10 ppb, THF-d_8: 7 ppb, C_6D_6: 15.5 ppb) was somewhat smaller in the hydrogen bond accepting solvents (DMSO-d_6, THF-d_8) than in C_6D_6 (Figure 10). Notably, in $CDCl_3$ containing 55% deuterated water, the isotopic multiplet displayed eight signals and an additional shoulder peak (Figure 11). This suggests that the $\boldsymbol{d_1}$/$\boldsymbol{d_3}$ and $\boldsymbol{d_0}$/$\boldsymbol{d_4}$ pairs of species which are isochronous in acetone-d_6, DMSO-d_6, THF-d_8, and C_6D_6, are anisochronous in $CDCl_3$. Since the sample was 55% deuterated, the population of the $\boldsymbol{d_3}$ species and $\boldsymbol{d_4}$ species are expected to be larger than the population of the $\boldsymbol{d_1}$ and $\boldsymbol{d_0}$ species, respectively. On the basis of their relative intensities, the pairs of signals "b" and "c" are assigned to $\boldsymbol{d_1}$ and $\boldsymbol{d_3}$ while signals "d" and "e" are assigned to $\boldsymbol{d_0}$ and $\boldsymbol{d_4}$. By exclusion, signal "f" is assigned to $\boldsymbol{d_2}$(***anti***) and $\boldsymbol{d_2}$(***distal***).

Temperature Effect on the Splitting of Pairs of Signals of the Isotopic Multiplet. The effect of lowering the temperature on the perturbation of the conformational equilibria was examined. The $\Delta\delta$ value for $\boldsymbol{d_3}$ and $\boldsymbol{d_1}$ in acetone-d_6 was 0.017 ppm at 298K, but this value increased to 0.019 ppm at 238 K. Similarly, the $\Delta\delta$ value of the pair of signals corresponding to the species doubly perturbed ($\boldsymbol{d_2}$(***syn***)) increased from 0.034 to 0.038 upon lowering the temperature to 238 K. These results indicate that the lower the temperature, the larger the relative population of the more stable form (i.e., the larger the perturbation of the conformational equilibrium). Further lowering the temperature may "freeze" the "in"−"out"/"out"−"in" conformational equilibrium depicted in Scheme 1. A sample of **2** in $CDCl_2F$[19] was cooled to 148 K, but no decoalescence of the two methoxy signals was observed. Apparently, although the intramolecular hydrogen bonds are expected to increase the rotational barriers of the "in"−"out" pair of methoxy groups, the rate of exchange is still too fast (on the NMR time scale) at 148 K.

Isotopic Multiplets in the OH Signal. In a ca. 50% deuterated sample of **2** in acetone-d_6, the OH signal appeared in the ^{1}H NMR spectrum as an isotopic multiplet resembling a quartet (Figure 1). This shape can be modeled, taking into

(19) Siegel, J. S.; Anet, F. A. L. *J. Org. Chem.* **1988**, *53*, 2629.

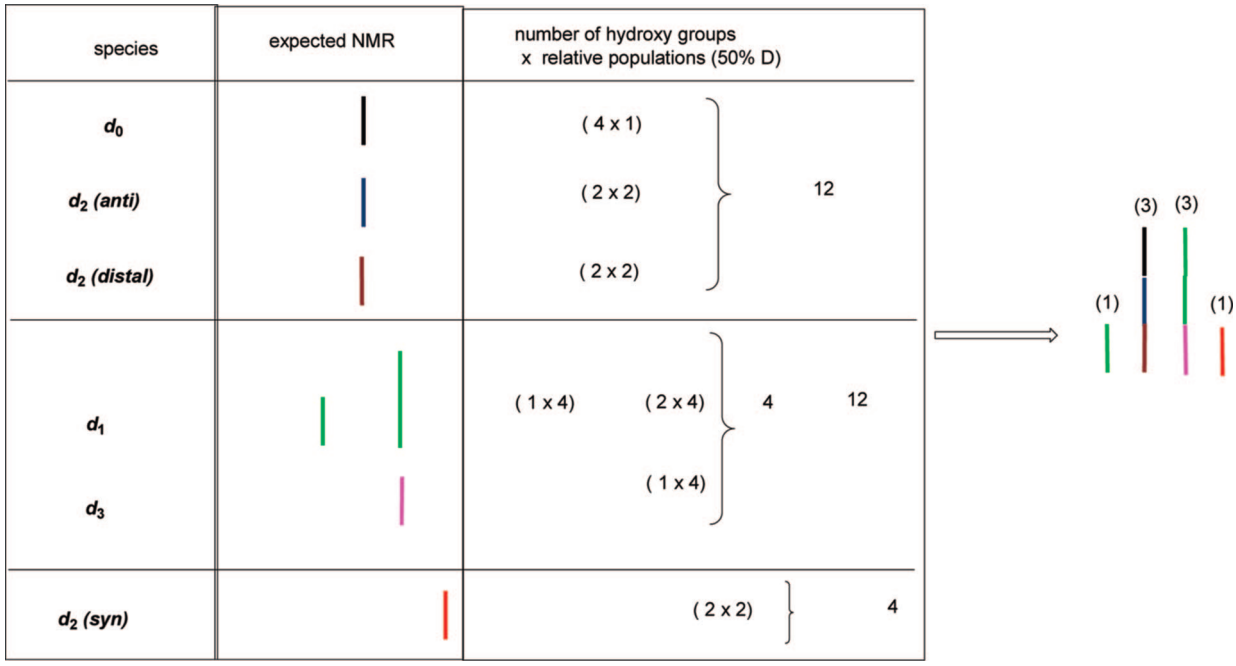


FIGURE 12. Assembling the isotopic multiplet of the hydroxyl group from the individual components. The numbers on the four signals of the isotopic multiplet resembling a quartet represent the expected relative integrations of the signals.

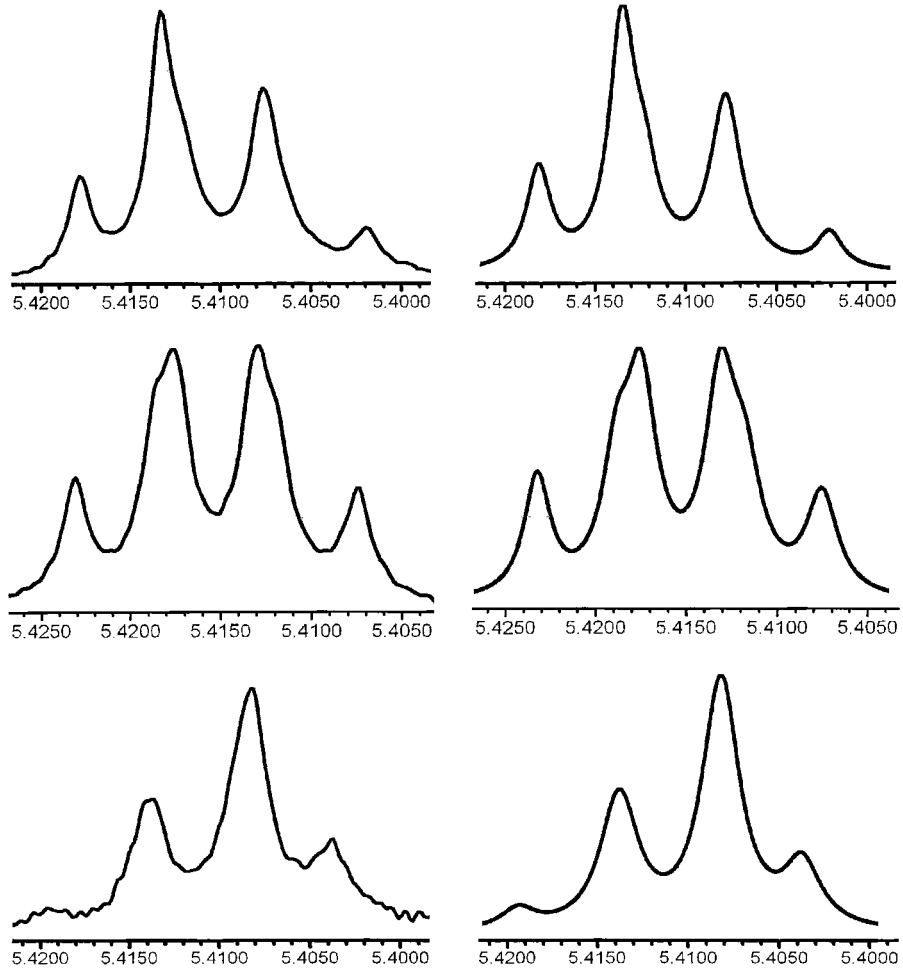


FIGURE 13. Experimental (left) and calculated (right) intensities of the isotopic multiplet of the OH group of **2** (in acetone-d_6). From top to bottom: 28%, 50%, and 76% deuterated sample. To reproduce the shoulders on the two central signals, slightly different chemical shifts were used for $\boldsymbol{d_0}$ and $\boldsymbol{d_2}$***(anti)*** + $\boldsymbol{d_2}$***(distal)*** and for $\boldsymbol{d_1}$/$\boldsymbol{d_3}$. Six signals were observed upon Gaussian resolution enhancement of the 50% deuterated spectrum (see the Supporting Information).

account the relative population of six of the seven forms (the ***d*****4** form is "silent" in the OH region of the ^{1}H NMR), the single (***d*****1** and ***d*****3** forms) or double isotopic (***d*****2**(***syn***)) perturbation of the equilibrium in three forms, and the number of OH groups present (Figure 12). The following points are assumed: (a) In **2**, a hydrogen bond of an OD to a methoxy group is stronger than a hydrogen bond of an OH group.[12b,20] On this basis, the conformations of ***d*****1**, ***d*****3**, and ***d*****2**(***syn***)) in which the methoxy group is hydrogen bonded to a larger number of OD groups (the three conformations at the left of Figure 7) are ascribed as the more stable forms. (b) Intramolecular hydrogen bonding results in a downfield shift. On this basis, the expected signals of ***d*****3** and ***d*****2**(***syn***) are drawn in Figure 12 upfield relative to the resonance of ***d*****0**. (c). The ***d*****0**, ***d*****2**(***anti***) and ***d*****2**(***distal***) forms are nearly anisochronous (d) The single signal of the ***d*****3** form is nearly isochronous to the downfield transition of the ***d*****1** form.

According to the assignment of the signals of a sample 50% deuterated, the two external signals of the isotopic multiplet of the OH signals correspond to different forms (upfield signal: ***d*****2**(***syn***), downfield signal: ***d*****1**). Thus, it could be expected that the mirror symmetry of the pattern of the isotopic multiplet should be destroyed when the percent of deuteration departs from 50%. For example, for a sample >50% deuterated it could be expected that the downfield external signal should decrease relative to the upfield external signal and conversely for a sample <50% deuterated. Indeed such a behavior was found experimentally, and as shown in Figure 13, excellent agreement was found between the experimental and calculated intensities of the isotopic multiplet.

Conclusions

The isotopic multiplets observed in the ^{1}H NMR spectrum of samples of **2** partially deuterated at the hydroxyl groups are due to a single and double isotopic perturbation of the "in"−"out"/"out"−"in" equilibrium of a pair of methoxy groups. These perturbations are the result of the different strengths of the intramolecular hydrogen bonds of the OH and OD group with a pair of methoxy groups. The different signals of the isotopic multiplet could be assigned to the different forms present in a partially deuterated sample and the general shape modeled taking into account the calculated population of each form and the number of protons contributed.

Experimental Section

Preparation of the Partially Deuterated Samples. In most cases, the NMR samples were prepared by dissolving **2** in commercial deuterated solvents (acetone-d_6, DMSO-d_6, C_6D_6, or $CDCl_3$) that were found to contain deuterium in the residual water. In the case of $CDCl_3$, or if a different deuteration enrichment was needed, small amounts of D_2O or H_2O were added by means of a capillary tube to solutions of **2**. The added D_2O together with the residual water of the solvents and the protons originating from **2** determine the percent deuteration at the OH groups. The resulting percent of deuteration was determined from the integration ratio of the H_2O and DOH signals (resonating in acetone-d_6 at ca. 2.805 and 2.760 ppm, respectively).

Simulations of the Spectrum of 2 in Acetone-*d*$_6$. The simulations of the spectra of the methoxy and hydroxy groups at different isotopic enrichments were conducted with the gNMR program.[21] For the spectra in acetone-d_6 it was assumed that (a) the ***d*****0**, ***d*****2**(***anti***), ***d*****2**(***distal***), and ***d*****4** forms are isochronous and display a single signal, (b) ***d*****1** and ***d*****3** are isochronous and display two signals, and (c) the ***d*****2**(***syn***) form displays two signals that have twice the separation of the signals of ***d*****1** and ***d*****3**. For the other simulations, the only isochrony assumed was between the ***d*****2**(***anti***) and ***d*****2**(***distal***) forms.

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Supporting Information Available: Parameters used for the simulation of the low-field MeO groups and OH groups of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO8020538

(20) Bolvig, S.; Hansen, P. E. *Curr. Org. Chem.* **2000**, *4*, 19.

(21) gNMR v4.1.0. Cherwell Scientific Publishing, Oxford, UK.