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Variable-Temperature Study of Hydrogen-Bond Symmetry in Cyclohexene-1,2-dicarboxylate Monoanion in Chloroform-*d*

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ABSTRACT

The symmetry of the hydrogen bond of hydrogen cyclohexene-1,2-dicarboxylate monoanion was determined in chloroform using the NMR method of isotopic perturbation. As the temperature decreases, the ^{18}O-induced ^{13}C chemical-shift separations increase not only at carboxyl carbons but also at ipso (alkene) carbons. The magnitude of the ipso increase is consistent with an ^{18}O isotope effect on carboxylic acid acidity. Therefore it is concluded that this monoanion is a mixture of tautomers in rapid equilibrium, rather than a single symmetric structure in which a chemical-shift separation arises from coupling between a desymmetrizing vibration and anharmonic isotope-dependent vibrations, which is expected to show the opposite temperature dependence.

INTRODUCTION

Hydrogen bonds (H-bonds) contribute to the shape and function of molecules such as water, proteins, and DNA. The principles of H-bonding are so basic that they are taught in every general chemistry course, yet so complex that they continue to be actively studied.[1]

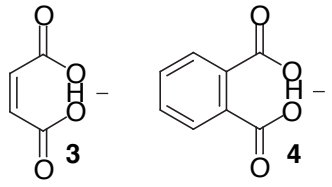
Hydrogen bonding is an attractive force between a proton donor A–H and a proton acceptor B. The attraction arises from a combination of interactions, including electrostatic, induction, electron delocalization, exchange repulsion, and dispersion.[2] For most H-bonds the primary contributor is electrostatic,[3,4] whereby the positive end of the A–H dipole stabilizes the negative charge on B. In addition, A and B must be similar in basicity to provide maximum stability to the H-bond.[5]

Symmetry of Hydrogen Bonds. A fundamental structural question is whether one or two minima exist on the potential-energy surface for motion of a hydrogen between two donor atoms. If there is one minimum, the hydrogen is centered between the two donor atoms, creating a symmetric H-bond **1**. If there are two, the hydrogen is at any instant closer to one of the atoms, resulting in an asymmetric H-bond **2a⇌ 2b** in a double-well potential.

—O- - -H- - -O— or —O–H- - - -O— ⇌ —O- - - - H–O—

1 **2a** **2b**

The monoanions of the dicarboxylic acids maleate **3** and phthalate **4** are classic examples that can show symmetric H-bonds. These ions exhibit characteristic features: a low barrier to hydrogen transfer, O–O distances of 2.4–2.5 Å,[6] a ^{1}H NMR signal around 20 ppm,[7] and fractionation factors greater than one for selectivity of deuterium over protium.[8] X-ray and neutron diffraction studies have shown that some crystals exhibit centered H-bonds,[9] although there are others that do not, owing to different environments surrounding the two carboxyls.[10]



3 **4**

When these low-barrier H-bonds are associated with both short donor separations and added strength, they are also called short, strong H-bonds. The basis for expecting a relation between distance and strength is largely due to one influential graph,[11] where the apparent correlation is due to the fact that all the very strong H-bonds are gas-phase, where ion-dipole forces are strong. The basis for expecting symmetric H-bonds to be strong may have arisen from viewing H-bonds as resonance hybrids (**2a ⟷ 2b**).[12] Maximum stabilization should occur when both resonance forms have identical energy, although symmetry is not guaranteed.[13] There has been

substantial interest in such H-bonds, owing to their proposed stabilization of intermediates or transition states in some enzyme-catalyzed reactions.[14]

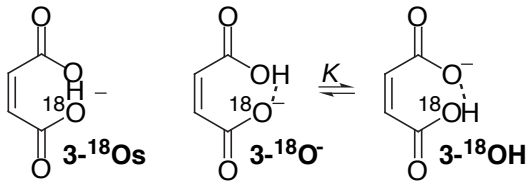
Isotopic Perturbation and Isotope Shifts. The NMR method of isotopic perturbation can distinguish symmetric structures from mixtures.[15] It is applicable to H-bonds. The method involves measurement of the isotope shift ${}^{n}\Delta$, defined as the chemical shift of a reporter nucleus X positioned *n* atoms away from a heavy isotope, relative to the chemical shift in the presence of the light isotope (Equation 1, with *n* sometimes omitted). It usually has a negative value,[16] including ^{18}O-induced ^{13}C NMR shifts.[17] In general, the observed isotope shift Δ consists of both an intrinsic shift Δ_0 and a shift Δ_{eq} induced by the perturbation of an equilibrium (Equation 2). The mere presence of an isotope is responsible for Δ_0,[18] while Δ_{eq} is due to differences in the mass-dependent vibrational frequencies and the ZPEs of the two species in equilibrium.[19] If the H-bond is symmetric, there is no equilibrium to perturb, and Δ must equal Δ_0.

$$ {}^{n}\Delta = \delta_X(\text{heavy}) - \delta_X(\text{light}) \quad (1) $$

$$ \Delta = \Delta_0 + \Delta_{eq} \quad (2) $$

This method can be illustrated with the monoanion of a mono-^{18}O-labeled dicarboxylic acid, such as ^{18}O-maleate (**3-^{18}O**). The ^{13}C NMR spectra of both the corresponding diacid and dianion show an ^{18}O-induced intrinsic shift, measured as the difference between the chemical shifts of ^{13}C–^{18}O and ^{13}C–^{16}O. These two intrinsic shifts happen to be equal. Moreover, the diacid is more shielded than the dianion. If the monoanion were present as a single symmetric structure (**3-^{18}Os**, in rapid equiilibrium with a second conformational isotopomer, of essentially the same energy, but with ^{18}O in the carbonyl), only an intrinsic isotope shift would be observed. In contrast, if there are two rapidly equilibrating tautomers (**3-^{18}O$^-$ ⇌ 3-^{18}OH**, each in rapid equiilibrium with a conformational isotopomer that has ^{18}O in the carbonyl), the isotopic substitution favors one tautomer over the other. Each of the observed chemical shifts is then a weighted average of the more shielded carboxylic-acid-like carbon and the more deshielded carboxylate-like carbon.

Consequently the $^{13}C–^{18}O$ and $^{13}C–^{16}O$ signals are separated by an additional Δ_{eq}. This method succeeds even when rapid equilibration coalesces NMR signals.



This method depends on the fact that isotopic substitution favors one tautomer over the other. The equilibrium constant K (= [**3-^{18}OH**]/[**3-^{18}O$^-$**]) is equal to K_a^{16}/K_a^{18} the ratio of the acidity constants of the ^{16}O carboxylic acid and the ^{18}O carboxylic acid. This is ~1.01, owing to ZPE differences.[20] As a result, the proton resides on the ^{18}O more often than on the ^{16}O, and the chemical shift of the $^{13}C–^{18}O$ resembles more that of the shielded diacid while the $^{13}C–^{16}O$ chemical shift resembles more that of the deshielded dianion.

Equation 3 relates Δ_{eq} to K and D, the difference between the chemical shifts δ_{COOH} and δ_{CO2-} of the carboxyl and carboxylate carbons in the monoanion. This parameter cannot be measured directly, but it can be approximated as the chemical-shift difference between the diacid and the dianion. (It should be noted that not only Δ_0 and Δ but also D and ΔG^o are < 0.) Then, by converting to a Gibbs-energy difference $\Delta G^o = -RT\ln K$ and expanding the exponential, Equation 2 becomes Equation 4. Therefore, if the perturbation of an equilibrium contributes to the observed isotope shift, then that isotope shift depends on temperature.

$$\Delta_{eq} = \frac{K-1}{K+1}\, D = \frac{K-1}{K+1}(\delta_{COOH} - \delta_{CO2-}) \tag{3}$$

$$\Delta = \Delta_0 - \frac{D}{2}\frac{\Delta G^o}{R}\frac{1}{T} \tag{4}$$

Previous Results. Isotopic perturbation, including the temperature dependence, was initially used by Saunders and coworkers to distinguish a mixture of equilibrating carbocations

from a static symmetric structure.[21] Those studies had the advantage of large chemical-shift differences (*D* in Equations 3-4) of up to 200 ppm between carbocationic and sp^3 carbons and consequently a large variation with temperature.

For many years Perrin and coworkers have been using isotopic perturbation to explore the symmetry of H-bonds. A wide range of dicarboxylate monoanions were found to show a small but significant Δ_{eq}, indicative of a mixture of tautomers,[22] even though these are symmetric in crystals and show single-well potentials according to high-level calculations.[23] The asymmetry was initially attributed to the polarity of aqueous solution, which stabilizes a localized negative charge more than a delocalized one,[24] Indeed, computer simulations supported that interpretation.[25] Yet a Δ_{eq} is detectable even in nonpolar organic solvents.[26] The asymmetry was therefore attributed more generally to the disorder of solvation and to the presence of solvatomers (isomers that differ in solvation).[27] Computer simulations support this interpretation too.[28] The asymmetry need not be restricted simply to a pair of tautomers, since it is also possible that the hydrogen is distributed across the O-O distance, with a structure determined by the instantaneous solvation.[29] In support of the role of solvation, it has recently been found that difluoromaleate monoanion too is asymmetric in aqueous solution and in dipolar apritic solvents but is symmetric in the crystal and in an isotropic liquid crystal phase.[30] Other examples show that asymmetry is not restricted to dicarboxylate monoanions but is also seen in N–H–N and N–H–O H-bonds.[31]

These results have been supported by other studies that found double-well potentials in succinate monoanions,[32] and in phenol-carboxylate complexes.[33] Even the proton-bound dimer of pyridine is asymmetric, despite a strongly deshielded ^{1}H NMR signal at δ 21.73.[34] Studies of homoconjugated anions of carboxylic acids, $(RCO_2)_2H^-$, at 110–120 K found that they are tautomeric, but that maleate and phthalate anions are symmetric, and the symmetrization was attributed to solvent ordering at these very low temperatures.[35]

In summary, we and others have been unable to find evidence for low-barrier H-bonds in solution (except at very low temperature).[35,36] If they were unusually strong, they ought to be more readily detectable. The lack of evidence for them implies that there is no substantial energetic

favorability associated with symmetric H-bonds, and that the disorder of solvation is sufficient to disrupt the symmetry that is calculated to characterize the isolated ion.[37] One of us has therefore deplored the common custom of considering short, low-barrier H-bonds as unusually strong.[38] In support of this conclusion, it was found that compression, to produce a "short, strong" H-bond, does not contribute to catalysis of enolization.[39] Moreover, a network of H-bonds can provide the stabilization to account for enzyme catalysis, rather than one short, strong H-bond.[40]

An Alternative Interpretation. Bogle and Singleton recently published an alternative interpretation of those NMR data that were presented as evidence for asymmetric tautomers.[41] They used gas-phase calculations of quasiclassical trajectories of hydrogen across the highly anharmonic potential-energy surface in isotopically labeled hydrogen phthalate anion and averaged the ^{13}C NMR shifts over those trajectories. They concluded that an ^{18}O can produce a significant intrinsic isotope shift, whose magnitude is sufficient to account for the results obtained by Perrin and coworkers. Then there is no need to propose equilibrating tautomers.

Bogle and Singleton carried out similar calculations on tetramethylbromonium ion, which Ohta and coworkers had assigned as asymmetric based on isotopic perturbation by CD_3 groups.[42] This ion, with its C–X^+–C, is similar to N–X^+–N species with halogen bonds,[43] which are found to be symmetric in CD_2Cl_2,[44] perhaps because both N–X bonds are fully covalent, as are the C–X bonds in a bromonium ion, and as distinct from O–H–O or N–H–N H-bonds. This controversy was resolved upon modeling the disorder of solvation in SO_2 and discovering that an oxygen from SO_2 adds to one carbon of the bromonium ion, producing an indisputably asymmetric species.[45]

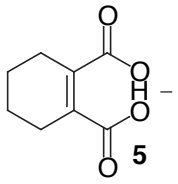
Proposal. We do not deny that the intrinsic isotope shift due to an ^{18}O can be substantial when there is coupling between a desymmetrizing mode and anharmonic isotope-dependent modes. The question remains whether this calculated isotope shift accounts fully for the isotope shifts we have measured.

An intrinsic isotope shift should be largely independent of temperature. In contrast, the dependence of isotope shift on temperature was key to Saunders' evidence for a mixture of carbocations.[21] Likewise, the carboxyl and ipso isotope shifts of aqueous hydrogen phthalate

monoanion decrease with increasing temperature, whereas the carboxyl isotope shift of phthalate dianion, which must be intrinsic, hardly varies with temperature.[22] Bogle and Singleton seem to accept the disorder of the water environment as strong enough to produce asymmetric ions, but they reject asymmetry in aprotic organic solvents,[41]

We therefore must evaluate the temperature dependence of an isotope shift in an aprotic organic solvent. If the isotope shift is due to perturbation of an equilibrium, it ought to increase at lower temperature. If the isotope shift is due to the desymmetrizing effect of isotopic substitution on a symmetric H-bond, then we infer that it is not necessarily temperature-independent, as asserted above. Instead we expect that it will decrease at lower temperature, because the vibrational amplitudes will decrease and vibrations will become more harmonic.

The goal of this work is to measure the ^{18}O-induced isotope shifts at the carboxyl and ipso positions (properly designated as alkene, but ipso preserves parallelism to previous studies) in the ^{13}C NMR spectrum of ^{18}O-labeled hydrogen cyclohexene-1,2-dicarboxylate **5**. Monoanion **5** was chosen because it had been found to exhibit a large perturbation shift in water.[26] The experiments are run at a series of low temperatures in chloroform-*d*, with the tetrabutylammonium salt for solubility. We now report that the ^{18}O-induced isotope shift in cyclohexene-1,2-dicarboxylate monoanion **5** is larger at lower temperature.



5

EXPERIMENTAL SECTION

Instrumentation. All mass spectral data were obtained using ESI-MS, negative ion mode on a Thermo LCQdeca-MS spectrometer. NMR spectra were obtained on a JEOL ECA500 FT-NMR spectrometer (500.2 MHz ^{1}H, 125.8 MHz ^{13}C) with $CDCl_3/CHCl_3$ as internal standard.

Synthesis of 5-$^{18}O_{0–4}$. A mixture of ^{18}O isotopologues of cyclohexene-1,2-dicarboxylic

acid **6** was synthesized by combining the anhydride with $H_2{}^{18}O$ and anhydrous THF (to increase solubility). The reaction mixture was stirred at room temperature for 20–24 hours. The extent of hydrolysis was monitored by thin-layer chromatography. The solid tetrabutylammonium salt of the monoacid monoanion **5** was then obtained by adding 1 equivalent of tetrabutylammonium hydroxide to **6** and removing the solvent.

Negative-ion mass spectrometry of ^{18}O-labeled diacid **6** was used to measure the ^{18}O content and distribution of ion **5**. The values are presented in Table 1, expressed as $P(n)$, the fraction with $n = 0$, 1, 2, 3, or 4 ^{18}Os. Although each preparation produced slightly different ratios, they were all very similar, and this table is representative.

Table 1. Masses and fractional amounts of $^{18}O_{0–4}$ isotopologues of diacid **6**.

m/*z*	$n(^{18}O)$	$P(n)$
169.17	0	0.089
171.17	1	0.422
173.17	2	0.366
175.16	3	0.117
177.20	4	0.006

NMR Sample Preparation. NMR samples were prepared as 0.1 M **5-$^{18}O_{0–4}$** in $CDCl_3$, and the presence of **5** was confirmed by ^1H NMR.

RESULTS

^{18}O-Induced ^{13}C NMR Isotope Shifts of Diacid 6. A ^{13}C NMR spectrum of the diacid **6**-$^{18}O_{0-4}$ in $CDCl_3$ shows chemical-shift separations of 26 ppm, 49 ppm, and <5 ppm for the monosubstituted carboxyl, disubstituted carboxyl, and ipso signals, respectively. Because there is no tautomeric equilibrium possible in the diacid these must be intrinsic isotope shifts. These values thus serve to calibrate expected values of intrinsic isotope shifts at carboxyl and ipso carbons.

^{18}O-Induced ^{13}C NMR Isotope Shifts at Carboxyl Carbons of Monoanion 5. The ^{1}H NMR evidence for **5** is presented in Figure S1 of the Supporting Information. Figure 1 shows the ^{13}C NMR signals at 293.9 K for the carboxyl carbons in a mixture of ^{18}O-labeled isotopologues of **5** with 1.0 Hz additional line broadening. Signals can be assigned tentatively as unlabeled ($\mathbf{A_0}$), mono-^{18}O-labeled ($\mathbf{A_1}$), and di-^{18}O-labeled ($\mathbf{A_2}$), based on the general result that an intrinsic isotope shift due to a heavy atom is shielding.[16] This assignment was confirmed by the addition of authentic unlabeled **5**, which increased the $\mathbf{A_0}$ intensity. Moreover, the relative intensities are consistent with the ^{18}O distribution in Table 1.

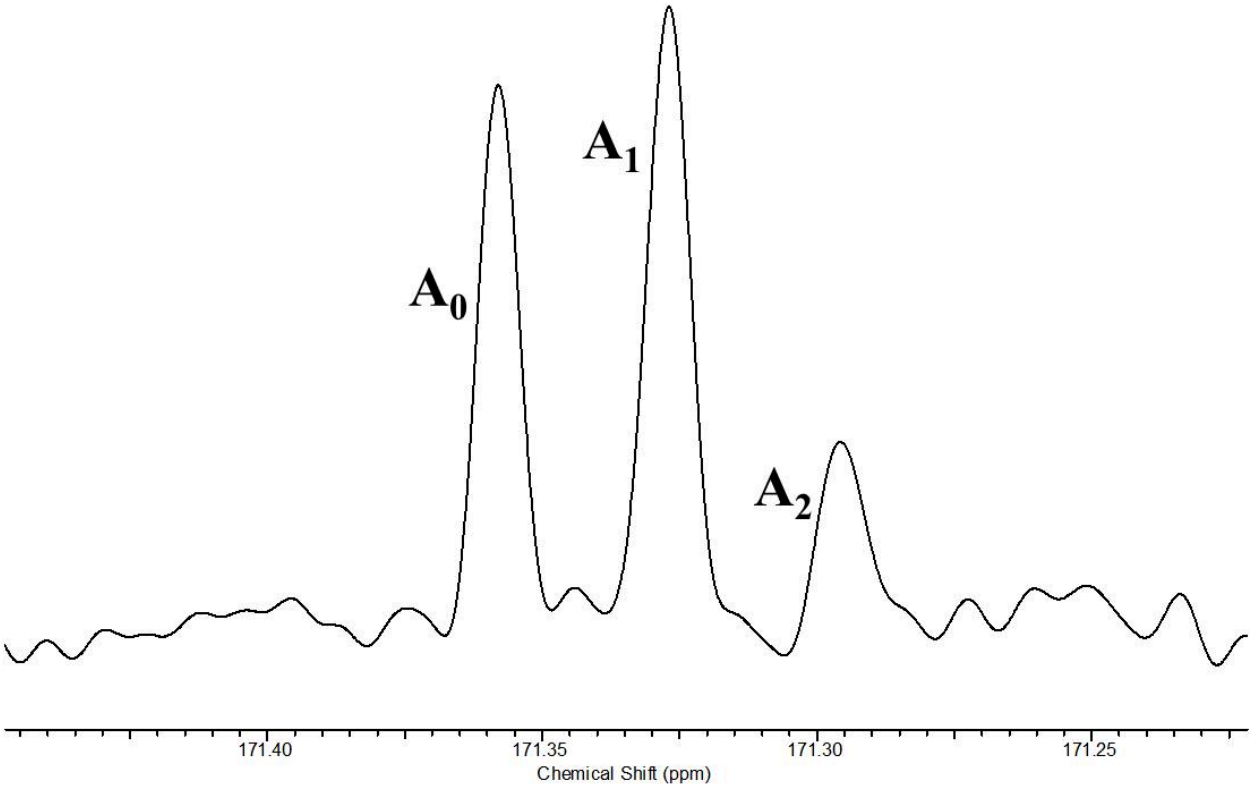


Figure 1. ^{13}C NMR spectrum of the carboxyl region of a mixture of ^{18}O-labeled isotopologues of **5** in $CDCl_3$ at 293.9 K with 1.0 Hz line broadening.

At increased resolution—and with no applied line broadening—the three signals of **5-$^{18}\mathbf{O_{0–4}}$** in Figure 1 separate into additional signals, as shown in Figure 2. The fine structure arises

from a four-bond isotope shift due to ^{18}O in the carboxyl on the opposite side of the ion. The signals are designated so that the first subscript is the number of ^{18}Os attached to a carboxyl carbon, as in Figure 1, while the second subscript represents the number of ^{18}Os attached to the other carboxyl carbon, which is responsible for the additional splitting in Figure 2.

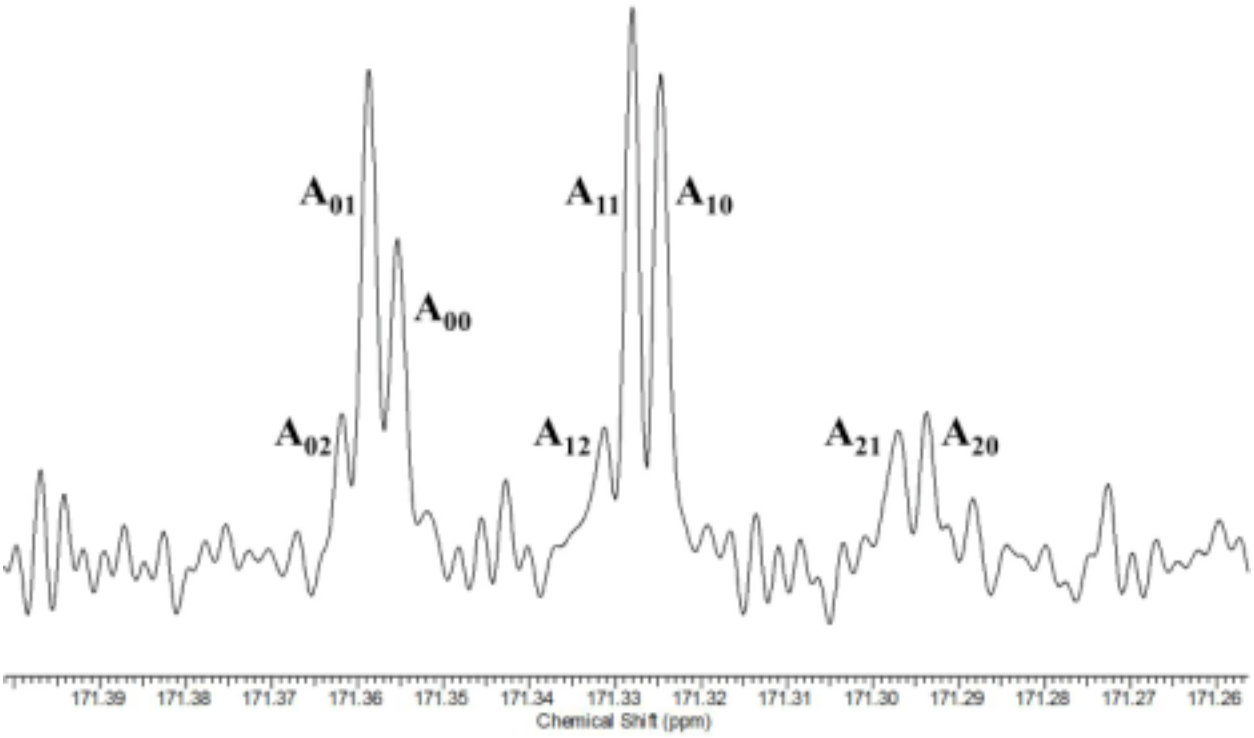


Figure 2. ^{13}C NMR spectrum of the carboxyl region of a mixture of ^{18}O-labeled isotopologues of **5** in $CDCl_3$ at 293.9 K with no applied line broadening.

Figure 3 shows all six possible ^{18}O-labeled isotopologues of **5**, including two distinct isotopomers with two ^{18}O labels.[46]. Isotopologues **5-$^{18}O_1$**, **5-$^{18}O_{2s}$**, and **5-$^{18}O_3$**, which have carboxyl groups with one ^{16}O and one ^{18}O, exist as a 1:1 mixture of two rapidly equilibrating conformational isotopomers, but for brevity only the one with ^{18}O involved in the H-bond is shown, and the justification for this simplification is presented in the Supporting Information. The correspondence between these six species and the signals in Figure 2 is justified in the Supporting Information, where it is shown that the relative intensities are consistent with the probabilities

derived from the ^{18}O distribution in Table 1. The assignments were further supported by the addition of authentic unlabeled monoanion, which resulted in an increase in the intensity of the **A_{00}** signal.

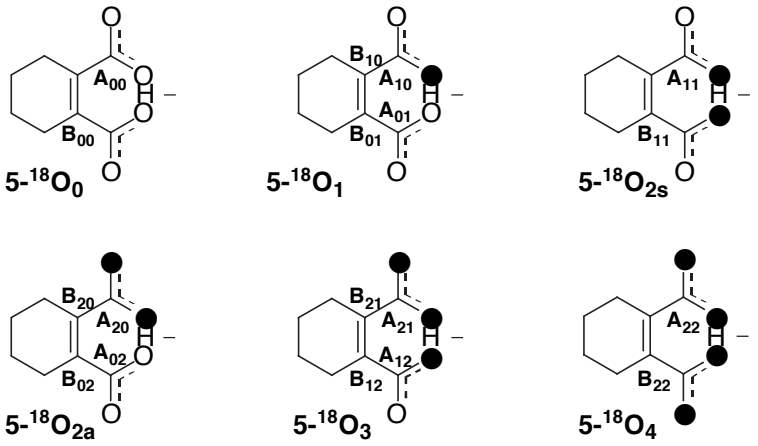


Figure 3. ^{18}O-Labeled isotopologues of **5** with 0, 1, 2, 3, or 4 ^{18}Os ("●") and designation of distinguishable carbons.

Temperature Dependence of Carboxyl Chemical Shifts. As the temperature decreases, the chemical shifts of the carboxyl carbons of ^{18}O-labeled **5** move apart, but only slightly, as can be seen in the spectra in Figure S2. At low temperatures the resolution deteriorates, so that the eight signals coalesce to three. Table S3 lists the chemical shifts of the 3 signals at lower resolution. Table S4 lists the chemical shifts of the 8 carboxyl signals at temperatures where the signals are resolvable.

To better reveal that temperature-dependence, isotope shifts Δ at the carboxyl carbons can be evaluated as differences between appropriate pairs of chemical shifts from Table S2. They, with their temperature dependence, are presented in Table 2. Because isotope shifts due to heavier atoms are negative, they are tabulated for simplicity as –Δ, the negative of the isotope shift. According to the structures in Figure 3, the chemical-shift difference –(**A_{11}** – **A_{00}**) is the negative of the sum of a one-bond isotope shift, $^{1}\Delta$, and a four-bond isotope shift, $^{4}\Delta$. Because there can be no

perturbation of a tautomeric equilibrium when both carboxyls have identical isotopic substitution, this sum must be an intrinsic shift, labeled as $^1\Delta_0+^4\Delta_0$. Similarly, according to the structures in Figure 3, the differences $-(\mathbf{A_{10}} - \mathbf{A_{01}})$ and $-(\mathbf{A_{21}} - \mathbf{A_{12}})$ represent $-(^1\Delta - ^4\Delta)$, while $-(\mathbf{A_{20}} - \mathbf{A_{02}})$ represents $-2(^1\Delta - ^4\Delta)$. These three are written without subscripts because they are not necessarily intrinsic shifts.

Table 2. Chemical-shift differences and ^{18}O-induced isotope shifts (ppb) for the carboxyl signal of ^{18}O-labeled **5** in $CDCl_3$.

Difference	Type	293.9 K	264.1 K	254.1 K
$-(\mathbf{A_{11}} - \mathbf{A_{00}})$	$-(^1\Delta_0 + ^4\Delta_0)$	27.3	28.4	28.8
$-(\mathbf{A_{10}} - \mathbf{A_{01}})$	$-(^1\Delta - ^4\Delta)$	34.0	35.8	35.9
$-(\mathbf{A_{21}} - \mathbf{A_{12}})$	$-(^1\Delta - ^4\Delta)$	34.2	35.8	36.9
$-(\mathbf{A_{20}} - \mathbf{A_{02}})$	$-2(^1\Delta - ^4\Delta)$	68.1	71.2	72.5

^{18}O-Induced ^{13}C NMR Isotope Shifts at Ipso Carbons of Monoanion 5 and Chemical-Shift Assignments. Figure 4 shows five ^{13}C NMR signals for the ipso carbons of the ^{18}O isotopologues of **5** at room temperature, with an applied line broadening of 1 Hz. The ipso (**B**) labeling nomenclature is the same as that used for the carboxyl carbon (**A**) in Figure 3. The first subscript represents the number of ^{18}Os on the carboxyl carbon adjacent to the ipso carbon of interest, and the second subscript represents the number of ^{18}Os on the opposite carboxyl. The justification for these assignments is presented in the Supporting Information. It depends on the agreement between the relative intensities in Figure 4 and the intensities calculated from the mass-spectrometric data in Table 1, divided among the contributions from each of the ipso carbon types of Figure 3, as presented in Table S5.

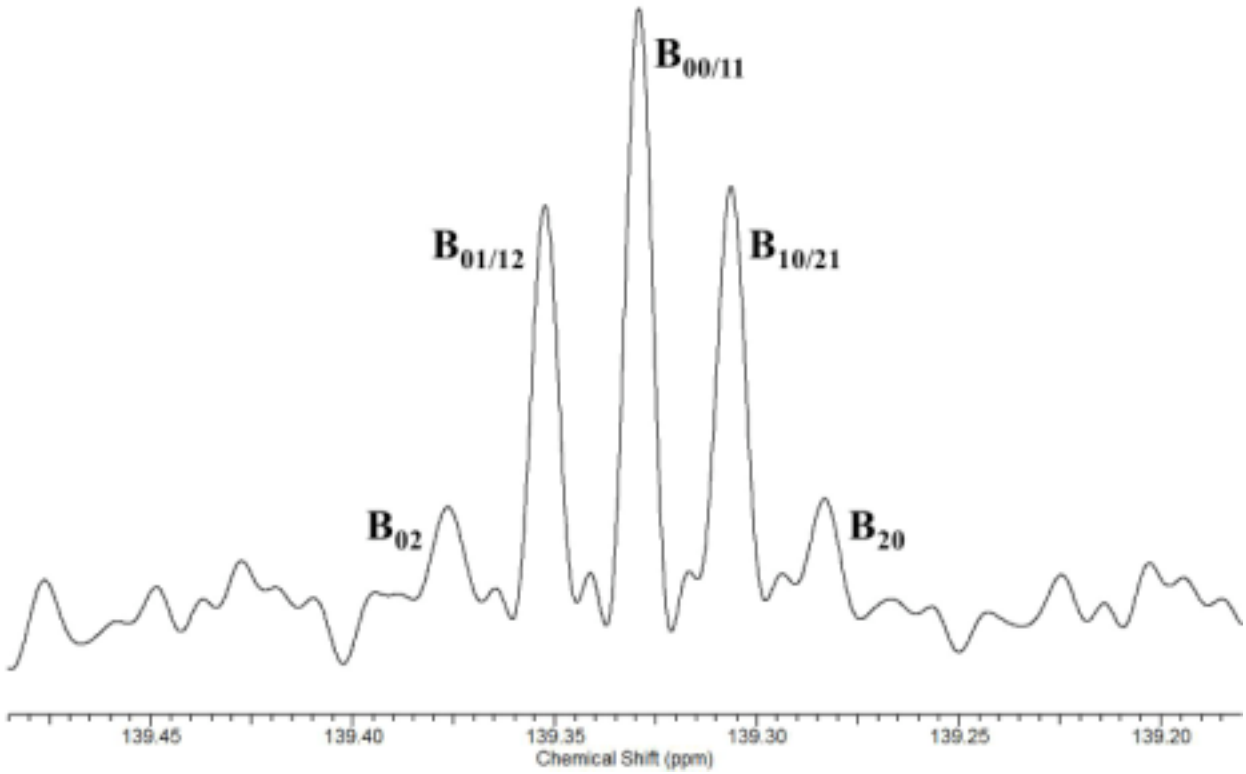


Figure 4. ^{13}C NMR signals of the ipso carbons of the ^{18}O isotopologues of **5** in $CDCl_3$ at room temperature, with an applied line broadening of 1 Hz.

The widths of the signals in Figure 4 are ≤ 3 ppb. Thus $\mathbf{B_{00}}$ and $\mathbf{B_{11}}$ appear as one signal and are designated as $\mathbf{B_{00/11}}$. The same is true for $\mathbf{B_{01}}$ and $\mathbf{B_{12}}$ as well as for $\mathbf{B_{10}}$ and $\mathbf{B_{21}}$, and they are designated as $\mathbf{B_{01/12}}$ and $\mathbf{B_{10/21}}$, respectively. The (unresolvable) chemical-shift separation $\mathbf{B_{11} - B_{00}}$ represents the sum of ^{18}O-induced isotope shifts $^2\Delta$ and $^3\Delta$. However, as with $\mathbf{A_{11} - A_{00}}$ this is an isotope shift between two structures with identical isotopic substitutions at both carboxyls and with no tautomeric equilibrium to perturb. This must therefore be an intrinsic isotope shift, $^2\Delta_0 + {}^3\Delta_0$. Then, because there is no resolvable difference between the chemical shifts of $\mathbf{B_{11}}$ and $\mathbf{B_{00}}$ within the signal labeled $\mathbf{B_{00/11}}$, this intrinsic isotope shift is negligible, just as in diacid **6** and as expected from phthalate monoanion **4**.[22] Moreover, the width of 3 ppb is an upper bound for this intrinsic isotope shift .

Temperature Dependence of Ipso Chemical Shifts. Similar to the carboxyl shifts, the

chemical shifts of the ipso carbons of ^{18}O-labeled **5** move apart as the temperature decreases. Figure 5 shows the variations, which are much more apparent than those of the carboxyl shifts.. Table S6 lists the chemical shift of each signal at various temperatures. Table 3 lists differences between the chemical shifts in Table S6, relative to that of the center signal, $\mathbf{B_{00/11}}$. These can be assigned as two-bond and three-bond isotope shifts, as indicated in Table 3. The differences $\mathbf{B_{01/12} - B_{10/21}}$ and $\mathbf{B_{02} - B_{20}}$ between ipso carbons in the same ion are also included in the table. These latter represent differences between the two-bond ($^2\Delta$) and three-bond ($^3\Delta$) isotope shifts. We next consider whether these isotope shifts represent an intrinsic shift, a perturbation shift, or a combination of the two.

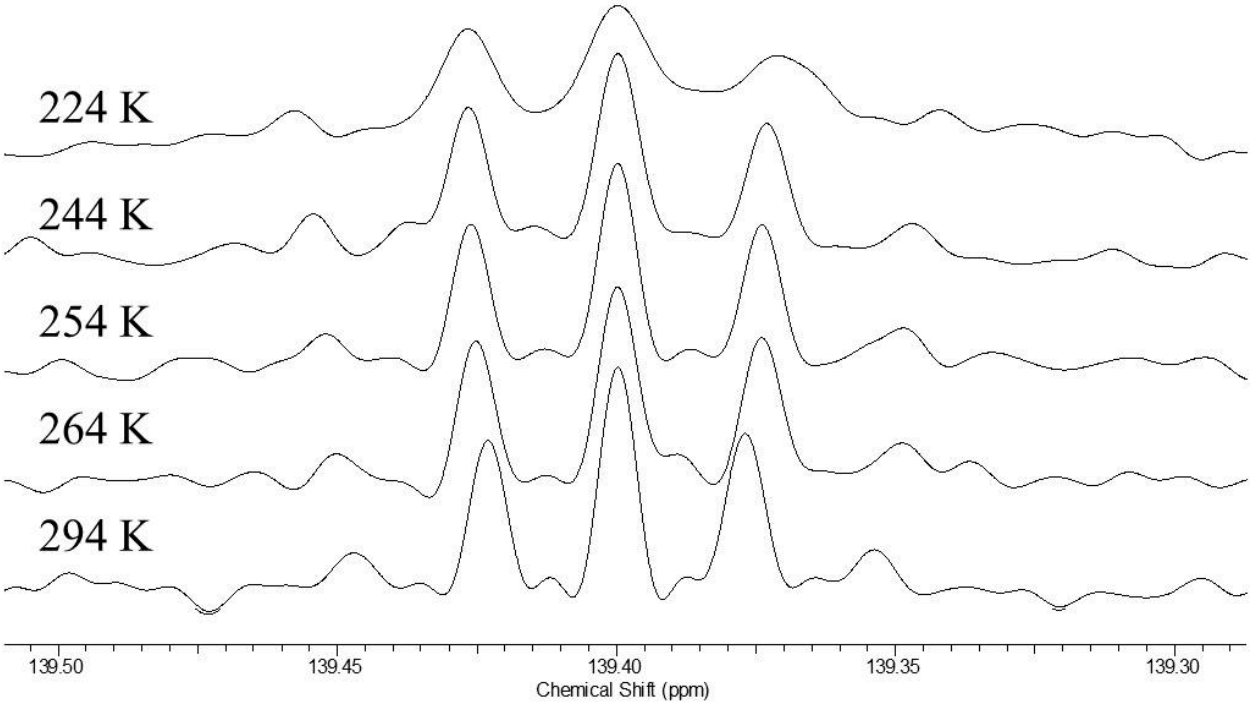


Figure 5. Temperature dependence of the ^{13}C NMR signals of the ipso carbons in ^{18}O isotopologues of **5** with an applied line broadening of 1 Hz.

Table 3. Temperature dependence of chemical-shift differences (ppb) of ipso carbons of ^{18}O-labeled **5** in $CDCl_3$, relative to the $\mathbf{B_{00/11}}$ signal, along with chemical-shift differences between carbons in the same ion.

Difference	Type	293.9 K	264.1 K	254.1 K	244.2 K	224.3 K
$-(\mathbf{B_{10/21} - B_{00/11}})$	$-^2\Delta$	22.8	25.8	25.8	26.7	28.6
$\mathbf{B_{01/12} - B_{00/11}}$	$^3\Delta$	23.2	25.4	26.3	26.8	26.8
$-(\mathbf{B_{20} - B_{00/11}})$	$-2^2\Delta$	46.0	51.0	51.2	52.8	57.6
$\mathbf{B_{02} - B_{00/11}}$	$2^3\Delta$	47.2	50.3	52.1	54.5	57.9
$\mathbf{B_{01/12} - B_{10/21}}$	$-(^2\Delta - {}^3\Delta)$	46.0	51.1	52.1	53.5	55.4
$\mathbf{B_{02} - B_{20}}$	$-2(^2\Delta - {}^3\Delta)$	93.2	101.3	103.4	107.3	115.5

If the isotope shifts in Table 3 were entirely intrinsic, they would remain essentially constant when the temperature is changed. Because they do vary, these isotope shifts must be due to perturbation of an equilibrium. Indeed, it was concluded above that the intrinsic isotope shift is negligible. The alternative possibility, that the two intrinsic isotope shifts $^2\Delta_0$ and $^3\Delta_0$ have opposite signs and nearly identical magnitudes, was rejected as unlikely. In contrast, isotope shifts $^2\Delta$ and $^3\Delta$ arising from perturbation of an equilibrium can have opposite signs and nearly identical magnitudes because the isotopic perturbation shifts the two carbons in opposite directions.

Equation 4 expresses the temperature dependence of an isotope shift due to perturbation of an equilibrium. A linear plot of the ipso isotope shifts Δ ($= {}^3\Delta - {}^2\Delta$ or $2({}^3\Delta - {}^2\Delta)$) versus 1000/T is displayed in Figure 6. The slopes are 8.8 ± 1.2 and 21.0 ± 0.7, respectively. The intercepts are 17 ± 5 and 22 ± 3. The correlation coefficients *R* are 0.973 and 0.998, respectively. The good linearity and correlation coefficients close to unity support a temperature-dependent perturbation of an equilibrium.

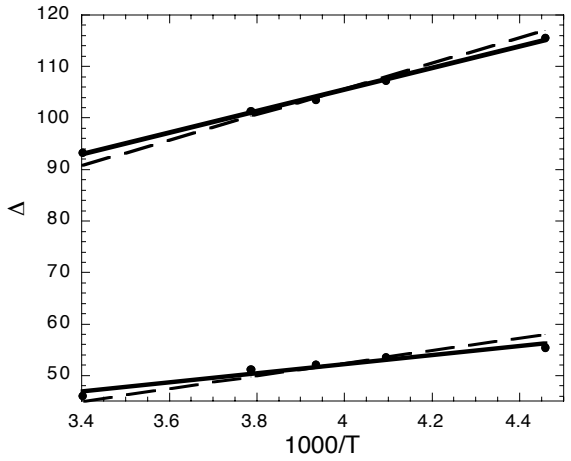


Figure 6. Linear fit (— two-parameter, -- one-parameter) of isotope shifts Δ vs 1000/*T* for the ipso carbons in the ^{18}O isotopologues of **5** in $CDCl_3$.

This plot also provides an estimate of the energy associated with the isotope effect on the equilibrium. With a reasonable estimate for the value of *D*, as defined in Equation 3, the slope in Figure 6 leads to a ΔG° of –5.1 cal/mol for $\mathbf{B_{01/12} - B_{10/21}}$ and –12.3 cal/mol for the $\mathbf{B_{02} - B_{20}}$ separation, corresponding to K_a^{16}/K_a^{18} values at 293 K of 1.009 and 1.011 per ^{18}O, respectively.

These ΔG° and K_a^{16}/K_a^{18} values are in good agreement with the typical ^{18}O isotope effect of ~1.01 on acidity.[20] However, the intercepts, 17 and 22 ppb, which ought to equal the intrinsic isotope shifts, are much higher than the maximum of 3 (or 6) ppb estimated from the width of the $\mathbf{B_{00/11}}$ signal. Moreover, the intercepts fail to differ by a factor of 2, and the errors in the intercepts are quite large.

This failure of the intercepts is a consequence of the inaccuracy in extrapolating the data to infinite temperature. To remedy this, the intercepts can be fixed at 3 and 6 ppb. One-parameter linear plots of the isotope shifts Δ with fixed intercepts are also displayed as the dashed lines in Figure 6. The slopes are 12.3 ±0.2 and 24.9 ±0.2, corresponding to K_a^{16}/K_a^{18} values per ^{18}O of 1.012 and 1.013, respectively, which are also reasonable.

DISCUSSION

Temperature Dependence of Carboxyl Carbon Isotope Shifts. Although the first entry of Table 2 is an intrinsic isotope shift that appears to increase from 293.9 K to 254.1 K, in the Supporting Information it is concluded that this increase is due to poor spectral resolution and that the intrinsic isotope shift is nearly constant. The other three entries in Table 2 are isotope shifts $-(^1\Delta - {}^4\Delta)$ in asymmetrically substituted ions. They are substantially larger than the intrinsic isotope shift. Therefore $^1\Delta$ and $^4\Delta$ must have opposite signs to make their difference smaller than their sum. This is consistent with perturbation of an equilibrium, which shifts the two signals in opposite directions.

Not only are these isotope shifts larger than the intrinsic isotope shift but also they show a greater dependence on temperature. While this variation with temperature is small, it does suggest that there is not only an intrinsic isotope shift, but also a perturbation of an equilibrium.

Temperature Dependence of Ipso Carbon Isotope Shifts. Although the increase of ^{13}C NMR isotope shifts with decreasing temperature is tenuous for the carboxyl carbons, it is quite firm for the ipso. Table 3 lists the ^{18}O-induced shifts for the ipso carbons of **5** at various temperatures in $CDCl_3$. The intrinsic isotope shift is $^2\Delta_0 + {}^3\Delta_0$, as might be measured from the separation between signals $\mathbf{B_{00}}$ and $\mathbf{B_{11}}$, which are due to symmetrically substituted ions. Unlike the carboxyl carbons, this separation is not resolvable because the width of the single $\mathbf{B_{00/11}}$ signal is < 3 ppb. We therefore conclude that the intrinsic isotope shift at the ipso carbons is negligible.

The absence of an intrinsic isotope shift implies that the chemical-shift differences, $\mathbf{B_{01/12} - B_{10/21}}$ and $\mathbf{B_{02} - B_{20}}$, between signals from asymmetrically substituted ions, are due to an isotope shift $^2\Delta - {}^3\Delta$ arising from perturbation of an equilibrium. This conclusion is further supported by the temperature dependence of the peak separations. As can be seen in Figure 5 and Table S6, the magnitude of $^2\Delta - {}^3\Delta$ increases as the temperature decreases. Moreoever, as can be seen in Figure 6, the dependence is adequately linear in $1/T$. These qualitative results support the conclusion that these isotope shifts arise from the perturbation of an equilibrium.

Not only are the isotope shifts larger at lower temperature but also the magnitude of the

temperature dependence is consistent with an origin in the perturbation of a tautomeric equilibrium. The slopes in the plots of Figure 6 can be converted to a Gibbs-energy difference ΔG^{o} and to an isotope effect on an equilibrium constant. The data from the two-parameter plots correspond to $K_a^{16}/K_a^{18} = 1.009$ and 1.011 per ^{18}O. These values are remarkably close to the 1.01 per ^{18}O generally observed for ^{18}O isotope effects on the acidity of a carboxylic acid.[20] Such a good quantitative agreement is fortuitous, but it is not as pertinent as the qualitative, order-of-magnitude agreement, owing to the uncertainty in *D* and the presence of dianion. Therefore the temperature dependence supports the attribution of the observed NMR isotope shifts to an ^{18}O-induced perturbation of an equilibrium between tautomers that differ in whether the proton is attached more firmly to an ^{18}O-labeled or unlabeled carboxyl.

Comparison of Carboxyl and Ipso Patterns. It may be puzzling that the carboxyl and ipso carbons show such different patterns. The pattern of the ipso carbons is symmetric about the $\mathbf{B_{00/11}}$ signal, but the carboxyl pattern is asymmetric. In essence this difference arises because the intrinsic shifts dominate the carboxyl signals whereas the perturbation shifts dominate the ipso signals. The central $\mathbf{B_{00/11}}$ signal is assigned to structures with identical isotopic substitutions at both carboxyls and with no tautomeric equilibrium to perturb. The other four ipso signals are paired. They are assigned to structures with unequal isotopic substitutions at their two carboxyls. The isotopic substitutions perturb the tautomeric equilibrium, favoring proton attchment to one carboxyl over the other, and shifting the components of each pair in opposite directions. In contrast, the carboxyl signals are split by the intrinsic isotope shift into three main signals, as in Figure 1, and with a small additional splitting from a perturbation shift, as seen in Figure 2.

Asymmetry of 5. The temperature dependence of the isotope shifts suggests that the dominant origin of those isotope shifts is the perturbation of an equilibrium by ^{18}O substitution. The only conceivable equilibrium is between tautomers that differ in whether the proton resides on the ^{18}O-labeled carboxyl or the unlabeled one. This is the same tautomeric equilibrium that was described in previous results from our laboratory. Although the H-bond is intrinsically symmetric, with a single-well potential, asymmetry arises from the disorder of solvation and the presence of

solvatomers. Thus we conclude that the H-bond in hydrogen cyclohexene-1,2-dicarboxylate monoanion **5** is asymmetric not only in water but also in chloroform.

CONCLUSIONS

According to the NMR method of isotopic perturbation, monoanion **5** has been found to exist in $CDCl_3$ as a mixture of tautomers, in an equilibrium that can be perturbed by ^{18}O substitution. The evidence is an isotope shift that is not merely intrinsic and that increases as the temperature decreases. In either of those tautomers the H-bond is asymmetric, with the proton more firmly attached to either the ^{18}O-labeled carboxyl or the unlabeled one. A symmetric H-bond would have only an intrinsic isotope shift, which is largely independent of temperature. We therefore dispute the conclusion that the isotope shift is only intrinsic,[41] and we reaffirm the conclusion that the monoanions of dicarboxylic acids such as **5** are asymmetric not only in aqueous medium but also in organic solvents.

We do not deny that the H-bond is intrinsically symmetric, with a single-well potential. Nor do we deny that coupling between a desymmetrizing mode and anharmonic isotope-dependent modes can contribute to the isotope shift. The question is whether this is the dominant contribution, or whether the dominant contribution is the perturbation by the instantaneous local environment of an equilibrium between tautomers. The temperature dependence that we observe suggests the latter.

Our results are consistent with an asymmetric H-bond, and we doubt that those results can be rationalized in terms of the desymmetrizing effect of isotopic substitution on a symmetric structure that is rendered asymmetric by coupling of anharmonic vibrations. The key question is whether the observed temperature-dependenc can be reproduced by calculations of the trajectory of hydrogen motion across the potential-energy surface of a hydrogen-bonded monoanion.[41] To the extent that lower temperature decreases the amplitudes of the motions and the mixing with anharmonic modes, we infer that the calculated isotope shift, although intrinsic, would not be temperature-independent but would decrease at lower temperature. If so, this would be inconsistent with our observation of a larger isotope shift at lower temperature. We therefore invite a calculation

of the temperature dependence of the isotope shift in hydrogen cyclohexene-1,2-dicarboxylate monoanion **5** or similar anion in an organic solvent. A computational counterpart to our experimental result is essential to answer this fundamental question about hydrogen-bond structure.

ASSOCIATED CONTENT

Supporting Information. Experimental methodology. Figures S1-S2. Justification for chemical-shift assignments. Tables S1-S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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46. Isotopomers are isomers that differ in the position of an isotope, whereas isotopologues differ in the number of isotopic subsititutions.

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