

## Chemical Shifts of Carbonyl Carbons in Peptides and Proteins

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**Abstract:** We report in this paper the results of an *ab initio* gauge-including atomic orbital study of the influence of hydrogen-bonding on the carbonyl carbon ( $^{13}\text{C}'$ ) chemical shift in peptides and protein model systems. For *N*-methylacetamide (NMA) interacting with formamide, the experimentally observed trends of the  $^{13}\text{C}'$  shielding tensor elements on hydrogen bond distance in peptides are moderately well reproduced. Shielding computations were also performed on SCF-optimized helical and  $\beta$ -turn *N*-formylpentaalaninamide structures. Here, we find the calculated helix–sheet chemical shift difference to be 4.9 ppm, with the helical site deshielded, in good agreement with experimental trends observed in proteins, where alanine  $^{13}\text{C}'$  helical sites are typically deshielded by  $\sim 4.6$  ppm when compared with sheet or sheetlike residues. The well-known  $^{13}\text{C}'$  helix–sheet chemical shift separation is therefore attributable to hydrogen bond formation, since  $\phi$ ,  $\psi$  effects alone (in model dipeptides) result in small upfield shifts for  $^{13}\text{C}'$  in helical sites. Unlike the situation with  $\text{C}^\alpha$ ,  $\text{C}^\beta$ ,  $\text{N}^{\text{H}}$ , and  $^{19}\text{F}$  shielding in proteins, *ab initio* geometry optimization of hydrogen-bonded systems appears to be essential in order to reproduce experimental shift patterns.

## Introduction

It is now known that there is an intimate relationship between the nuclear magnetic resonance (NMR) chemical shifts of the heavier nuclei ( $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{19}\text{F}$ ) in proteins and structure, making chemical shifts potentially powerful new structure probes.<sup>1</sup> For example, following the empirical work of Spera and Bax,<sup>2</sup> *ab initio* methods have recently been shown to be capable of predicting the dependencies of  $\text{C}^\alpha$  and  $\text{C}^\beta$  chemical shifts on the torsion angles  $\phi$  and  $\psi$ .<sup>1,3–6</sup> In addition, electrostatic field contributions to  $^{15}\text{N}$  and  $^{19}\text{F}$  chemical shifts in proteins have also been shown to be tractable via use of the multipole shielding polarizability<sup>7</sup> or charge-field perturbation approaches,<sup>1</sup> with van der Waals dispersion contributions to shielding being negligible.<sup>8</sup> However, among these successful interpretations of most heavy atom chemical shifts in peptides and proteins, the case of the carbonyl carbon,  $\text{C}'$ , has remained elusive. Empirically, it has been known for some time that  $\text{C}'$  chemical shifts are strongly correlated with secondary structure,<sup>9</sup> in much the same way that  $\text{C}^\alpha$  and  $\text{C}^\beta$  shifts are.<sup>1,2</sup> For example,  $\text{C}'$  sites in sheetlike alanine residues are, on the average, 4.6 ppm more shielded than their helical counterparts.<sup>10</sup> However, *ab initio* calculations, which take into account the influence of the torsion angles  $\phi$  and  $\psi$ , have shown just the opposite trend, with helical  $\text{C}'$  sites being more shielded.<sup>5,6</sup> Hence, the

secondary or structure-dependent shifts observed for these  $\text{C}'$  nuclei are likely due to factors other than the  $\phi$  and  $\psi$  torsion angles, and indeed this was pointed out in previous work by Jiao et al.,<sup>5</sup> who suggested hydrogen-bonding as a likely factor.

Early work done on peptides in solution<sup>11,12</sup> has also indicated that hydrogen-bonding causes a deshielding of  $\text{C}'$ . Thus,  $\alpha$ -helical hydrogen-bonding resulting in deshielding seems a plausible explanation for the observed shift patterns. The effects of hydrogen-bonding have also been investigated experimentally in the solid state, by Ando et al.,<sup>13,14</sup> who found a linear dependence between  $\text{C}'$  shielding and hydrogen bond length ( $R_{\text{N-O}}$ ). Moreover, semiempirical calculations within the finite perturbation theory–intermediate neglect of differential overlap (FPT-INDO) framework were also performed, which agreed qualitatively with the experimental trends. However, the agreement only became apparent when the range of hydrogen bond N–O distances was “adjusted”, and the calculated dependence was much weaker than the trend observed experimentally. In this work, we have reinvestigated, at the Hartree–Fock level, the effects of hydrogen-bonding on the  $\text{C}'$  chemical shift by using the gauge-including atomic orbital (GIAO) method.<sup>15–17</sup> Our results show clearly the importance of hydrogen bond interactions on  $\text{C}'$  shielding, both in simple model compounds and in pentapeptides, with both experimental protein  $\text{C}'$  shift range and sign now being correctly predicted.

## Computational Aspects

In the first part of this work we have investigated the nuclear magnetic resonance chemical shielding of the carbonyl carbon in *trans*-

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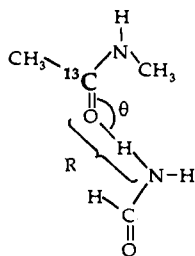
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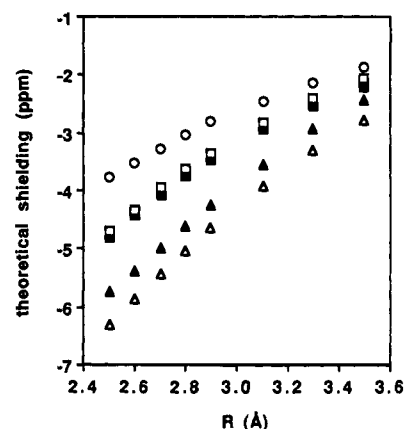
*N*-methylacetamide, interacting with a single formamide hydrogen bond partner:



This system was chosen for study since it is still amenable to use of large basis sets, which tend to minimize basis set superposition errors in supermolecule calculations, and counterpoise corrections to shielding derived by introducing ghost orbitals onto the formamide molecule were found to be negligible. Partial geometry optimizations at the SCF level were performed with a 6-311G\*\* basis at the following fixed values for the hydrogen bond angle,  $\theta$  (C=O $\cdots$ H): 120°, 150°, 180°, -150°, -120°. The hydrogen bond distance,  $R$  (N $\cdots$ O), was also varied as follows: 3.5, 3.3, 3.1, 2.9, 2.8, 2.7, 2.6, and 2.5 Å. Thus, there was a total of 40  $R$ ,  $\theta$  points. Shielding calculations were performed on the optimized structures using a 6-311++G(2d,2p) basis. We used the TEXAS90 program of Pulay, Wolinski, and Hinton<sup>18</sup> for both geometry optimization and shielding calculations, which were carried out on IBM (International Business Machines Corp., Austin, TX) RS/6000 workstations. Geometry optimization for a pair of  $R$ ,  $\theta$  values typically took 48 h, and a shielding calculation  $\sim$ 8 h, on an RS/6000 Model 360 computer. For the protein-model C' chemical shift computation, optimized structures for helical and  $\beta$ -turn *N*-formylpentaalaninamide fragments were obtained from the recent work of Schafer et al.<sup>19</sup> A 6-31G basis was used in the chemical shielding calculations, with polarization functions on the amide (C, O, N, H) atoms containing the C' site of interest. Shielding calculations here took about 18 h each.

## Results and Discussion

**Model Compound Studies.** We first sought to study the effects of hydrogen-bonding on C' shifts in the simple amide system shown above. Although there would be no direct comparison with a specific experimental data base, we felt the basic physics of the peptide hydrogen bond interaction should be exemplified in this system, and it was hoped that the experimental trends in the <sup>13</sup>C' shielding tensor element as a function of hydrogen bond length would be reproduced. Figure 1 shows the change in theoretical shielding for C' in *N*-methylacetamide as a function of hydrogen bond length ( $R_{N\cdots O}$ ) and orientation,  $\theta$ , where it can be seen that there is a considerable orientational dependence of the hydrogen bond effect. In a linear C=O $\cdots$ H-N system (O in Figure 1), the deshielding effect is at a minimum, and the influence of the hydrogen bond partner increases as the H-N bond becomes coincidental with the axis of either lone pair on oxygen. Solid-state measurements in more complex hydrogen-bonded alanines also show this dependence, as seen for example when comparing the alanine C' sites in *N*-acetyl-*N'*-methyl-L-alaninamide ( $\theta = 138^\circ$ ,  $R = 2.9$  Å,  $\delta = 175.9$  ppm) and *N*-acetyl-L-alanyl- $\alpha$ -aminoisobutyric acid methyl ester ( $\alpha = 154^\circ$ ,  $R = 2.9$  Å,  $\delta = 174.7$  ppm).<sup>14</sup> The former exhibits a hydrogen bond angle closer to 120°, and its C' chemical shift is more deshielded, in accord with the results shown in Figure 1. Also of considerable interest is the observation that a significant part of the calculated



**Figure 1.** Calculated isotropic chemical shielding dependence of <sup>13</sup>C' in *N*-methylacetamide-formamide dimer (isolated molecule 0 ppm) as a function of the hydrogen bond distance,  $R$  (O $\cdots$ N), for the following orientations,  $\theta$  (COH) = 120° ( $\blacktriangle$ ), 150° ( $\blacksquare$ ), 180° ( $\circ$ ), -150° ( $\square$ ), and -120° ( $\triangle$ ).

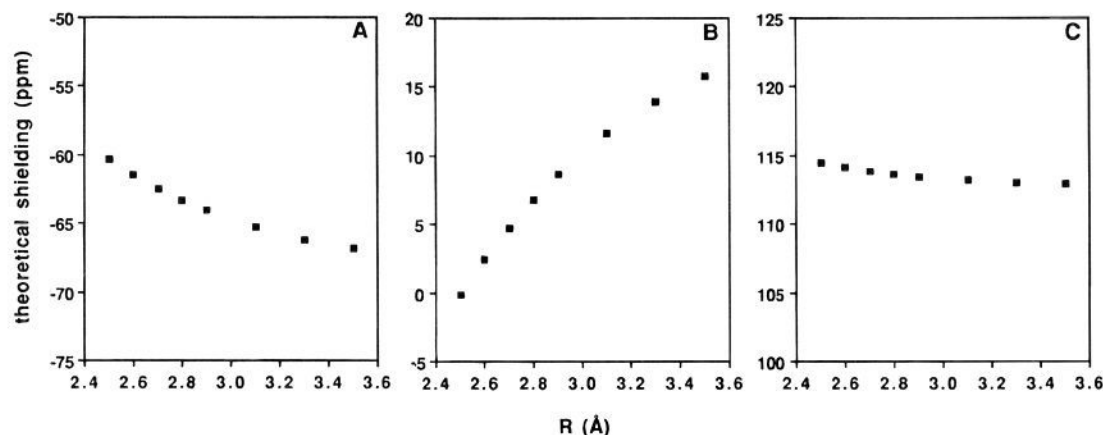
shielding dependence on hydrogen bond distance is found to be an *indirect* consequence of the presence of the perturbing formamide molecule, which is to say *it is due to a change in fragment structure*. For example, our results show that hydrogen-bonding causes large structural changes in the *N*-methylacetamide fragment, in particular the length of the C=O bond. This bond stretches as the hydrogen bond partner comes closer, with the C' shielding derivative with respect to this bond length being very substantial, -364 ppm/Å. At the closest hydrogen bond distance (2.5 Å,  $\theta = 120^\circ$ ), geometry optimization leads to a C=O bond length that is about 0.007 Å longer than in the absence of geometry optimization, and this accounts for a large (2.5 ppm) deshielding effect.

We next consider the behavior of the principal elements of the C' shielding tensor as a function of hydrogen-bonding. For *N*-methylacetamide alone, the C' absolute shielding is 22.79 ppm (the experimental isotropic shielding value obtained in a dimethyl sulfoxide solution is 18 ppm<sup>20</sup>), and the C' shielding tensor has the following principal elements: -71.27, 26.33, and 113.30 ppm. The calculated behavior of the principal elements of the C' shielding tensor for  $\theta = 150^\circ$  as a function of hydrogen-bonding are shown in Figure 2. The  $\theta = 150^\circ$  orientation is the one closest to the series of compounds studied by Asakawa et al.,<sup>14</sup> although the calculated tensor components for the other orientations all exhibit very similar behavior (data not shown). Figure 2A shows the hydrogen bond distance dependence of the most deshielded component,  $\sigma_{11}$ , which lies almost perpendicular to the C=O bond. This component becomes more shielded as the hydrogen bond partner comes closer, in accord with the behavior observed experimentally with alanine peptides.<sup>14</sup> However, the experimental shift of 8 ppm for  $R$  values ranging from 2.7 to 3.1 Å is larger than the calculated value of 3 ppm. The component which is most sensitive to the hydrogen bond interaction is  $\sigma_{22}$ , which lies approximately along the C=O bond. As shown in Figure 2B,  $\sigma_{22}$  decreases with decreasing  $R$ , as seen experimentally, and dominates the overall effect seen on the isotropic shielding, Figure 1. The change in  $\sigma_{22}$  from 2.7 to 3.1 Å is again small, about 6 ppm, versus an 18 ppm experimental shift in the alanine peptides, while  $\sigma_{33}$  (which lies perpendicular to the amide plane) is insensitive to hydrogen-bonding, as found experimentally.<sup>14</sup> These relatively small shielding changes can, however, be readily traced to our use of an unsubstituted fragment, since it

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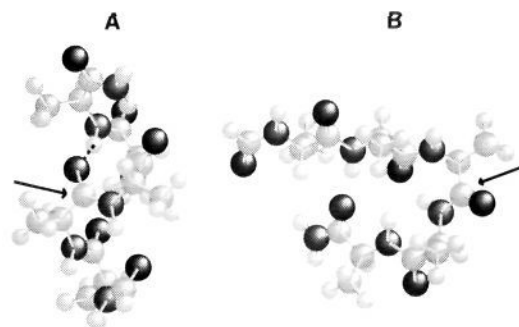


**Figure 2.** Calculated dependencies of the  $^{13}\text{C}'$  shielding tensor components in  $[^{13}\text{C}']$ -*N*-methylacetamide interacting with formamide, as a function of  $R_{\text{N-O}}$ , for  $\theta = 150^\circ$ : (A)  $\sigma_{11}$ ; (B)  $\sigma_{22}$ ; (C)  $\sigma_{33}$ .

is well known that  $\text{C}^\alpha$  substituents greatly affect the sensitivity of  $\text{C}'$  to hydrogen-bonding. For example, the chemical shift of  $\text{C}'$  in alanine residues is almost twice as sensitive to hydrogen-bonding as  $\text{C}'$  of glycine residues.<sup>14</sup> Nevertheless, the results presented in Figures 1 and 2 do provide a good theoretical basis for the experimentally observed dependencies of  $\text{C}'$  shielding on hydrogen bond distance *and* orientation, and do not require going to unrealistically short hydrogen bond distances in order to explain the experimentally observed trends, as done previously.<sup>14</sup>

**$^{13}\text{C}'$  Chemical Shift Problem in Proteins.** We believe that the results we have shown above give very strong clues as to the likely importance of  $\phi$ ,  $\psi$  and hydrogen-bonding effects on  $^{13}\text{C}'$  shielding in proteins. As mentioned previously, earlier attempts at solving this problem took into account only the  $\phi$  and  $\psi$  dependencies of  $\text{C}'$  shielding in proteins, and did not yield results in accord with experiment,<sup>5</sup> and from experimental work on small peptides in the solid-state<sup>13,14</sup> it can immediately be seen that  $\text{C}'$  shifts are only very mildly influenced by  $\phi$  and  $\psi$ —in sharp contrast to the situation found with, e.g.,  $\text{C}^\alpha$  and  $\text{C}^\beta$  shielding.<sup>1-3</sup> Indeed, all of the alanine  $\text{C}'$  chemical shifts described in the early peptide work, whether they correspond to helical or sheetlike  $\phi$  and  $\psi$  torsion angles, appear to be overwhelmingly dominated by hydrogen bond effects, suggesting to us that similar effects might dominate in proteins also.

To test this hypothesis, we first carried out an inspection of the torsion angles and hydrogen-bonding patterns in two proteins, staphylococcal nuclease<sup>21</sup> and a vertebrate calmodulin,<sup>22</sup> and we found that more than 90% of those residues which exhibit helical  $\phi$  and  $\psi$  torsion angles have their peptide  $\text{C}=\text{O}$  group hydrogen bonded, compared with only 30% of residues which have  $\psi > 50^\circ$ . Most of these 30% also have hydrogen bond angles close to  $170^\circ$ , which as can be seen from Figure 1 is expected to generate a much smaller effect on shielding than typical hydrogen bond angles in helices. Thus, simply by taking into account the presence of hydrogen-bonding, it appears likely that most of the helix-sheet  $^{13}\text{C}'$  chemical shift separation can be very simply interpreted as being due to the presence of hydrogen-bonding. There are of course additional effects arising from the torsion angles  $\phi$  and  $\psi$ , but from our computations on isolated dipeptide fragments (data not shown), a helical ( $\phi = -55^\circ$ ,  $\psi = -55^\circ$ ) fragment is shielded by only  $\sim 1$  ppm compared to either a sheetlike fragment ( $\phi = -140^\circ$ ,  $\psi = 140^\circ$ )



**Figure 3.** Molecular models of *N*-formylpentaalaninamide in (A) helical and (B)  $\beta$ -turn motifs, showing the location of  $^{13}\text{C}'$  sites. The Ala<sup>2</sup> site is hydrogen-bonded in (A). Ala<sup>3</sup> is not hydrogen-bonded in (B). The structures are based on those reported in ref 19.

or a  $\beta$ -turn fragment ( $\phi = -80^\circ$ ,  $\psi = 69^\circ$ ), and this small effect is simply overwhelmed by hydrogen-bonding.

To further explore the effects of hydrogen-bonding on  $\text{C}'$  chemical shifts in proteins, we then carried out shielding calculations on two model alanine pentapeptides. In recent work by Schafer et al.,<sup>19</sup> full geometry optimizations were performed on *N*-formylpentaalaninamide at various conformations: helix,  $\text{C}^7$ ,  $\text{C}^5$  and a  $\beta$ -turn. The shielding values calculated for the  $\text{C}'$  sites for Ala<sup>2</sup> in the helical conformer ( $\phi = -64.0^\circ$ ,  $\psi = -17.6^\circ$ ), Figure 3A, and on the non-hydrogen-bonded Ala<sup>3</sup> in the  $\beta$ -turn conformer ( $\phi = -79.9^\circ$ ,  $\psi = 68.8^\circ$ ), Figure 3B, are found to be 24.9 and 29.8 ppm, respectively. These results show that the hydrogen-bonded residue with helical  $\phi$  and  $\psi$  torsion angles has its  $\text{C}'$  site deshielded by 4.9 ppm, in excellent agreement with the survey of  $\text{C}'$  shifts,<sup>10</sup> which shows helical alanine  $\text{C}'$  nuclei to be typically deshielded by about 4.6 ppm when compared with sheetlike residues.<sup>10</sup> In this particular pair of molecules, the  $\phi$ ,  $\psi$  only contribution to shielding deduced from *N*-formylalaninamide model compounds<sup>3</sup> is  $-1.3$  ppm, which corresponds to a hydrogen bond contribution to shielding of 6.2 ppm. Our results therefore indicate that the  $^{13}\text{C}'$  helix-sheet chemical shift separation is not directly caused by  $\phi$ ,  $\psi$  effects, but is only indirectly related, due to the presence of strong hydrogen-bonding (in the  $\alpha$ -helix). For such highly polarizable groups, it is also possible that longer-range electrostatic field effects in proteins, as well as correlation effects, may influence shielding somewhat, but the dominant  $\text{C}'$  helix-sheet chemical shift separation is already well accounted for by hydrogen bonding.

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### Conclusions

The results we have presented above give the first satisfactory account of the  $^{13}\text{C}$  helix-sheet chemical shift separation found in proteins.<sup>10</sup> Our results strongly suggest that the experimentally determined helix-sheet chemical shift separations of  $\sim 4\text{--}5$  ppm do not originate from direct  $\phi$ ,  $\psi$  effects, which cause the helices to be somewhat shielded, but rather are dominated by strong peptide carbonyl-HN hydrogen-bonding in the  $\alpha$ -helices. While  $\text{C}^\alpha$ ,  $\text{C}^\beta$ , and  $\text{C}'$  chemical shifts all give useful information

on secondary structure,<sup>1-3,10</sup> there are fundamental differences in the origins of  $\text{C}'$  versus  $\text{C}^\alpha$  and  $\text{C}^\beta$  shifts, with  $\text{C}'$  shifts being only indirectly related to  $\phi$  and  $\psi$ .

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