

Calculation of trans-Hydrogen-Bond ^{13}C – ^{15}N Three-Bond and Other Scalar J -Couplings in Cooperative Peptide Models. A Density Functional Theory Study

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Abstract: We report B3LYP DFT calculations on peptide models that consider the effects of cooperative interactions with proximate H-bonds and local geometry at the H-bonding site upon trans-H-bond ^{13}C – ^{15}N three-bond scalar J -couplings. The calculations predict that cooperative interactions with other H-bonds within a H-bonding chain can significantly increase the magnitude of these couplings. Such increases are due to a combination of the presence of the neighboring H-bonds and the slight increase in C=O distances expected for peptide H-bonds near the centers of H-bonding chains. The energies of H-bonds inferred from H-bonding distances, alone, could be significantly in error if the effects of neighboring H-bonds are ignored.

Introduction

There have been many recent major advances in the study of protein and peptide structures. Since the first reports of trans-H-bond scalar ^3H -couplings in nucleic acids¹ and proteins,^{2–4} the measurement of these J -couplings in proteins has emerged as an important new technique used for the study of such structures.^{5–13} These couplings allow for direct measurements of the H-bond topology and the local structural details around the individual H-bonds in complex peptides. Several papers have appeared that correlate these couplings with the H-bonding distances and angles involved in such H-bonds.^{3,5,12} Theoretical studies have been published that correlate calculated and measured couplings and that predict couplings as a function of H-bond distance and related valence and torsional angles.¹² For the most part, these studies have relied upon the important contribution of the Fermi contact (FC) term to the overall coupling constant, as only this term has been calculated in most

of these studies. In addition, the calculations on couplings within large peptides have been simplified by extracting amide dimers from the larger structures, terminating their valences with hydrogens, and optimizing the positions of the H's. Bagno also used amino acids terminated with acetyl groups.⁵ The locations of the H-bonding H's were also determined by geometric optimization as their positions are not accurately given by the crystallographic data used. These approximations have been justified by earlier reports.¹⁴

The many more reports of calculated NMR coupling constants on more traditional systems have been reviewed,¹⁵ most recently by Contreras et al.¹⁶ Such coupling constants are among the most difficult molecular observables to accurately calculate. Even increasing the complexity to very large basis sets often does not lead to satisfactory conversion to consistent or observed results.¹⁷ Despite this, most of the theoretical calculations on trans-H-bond couplings have (necessarily, due to their complexity) been performed with small-to-moderate basis sets. The results have been *correlated* with experiment (they do not necessarily match experiment). We report elsewhere that the ^{13}C – ^{15}N three-bond scalar J -couplings (as well as three other trans-H-bond J 's) are not as sensitive to basis sets¹⁸ as some other J 's previously studied by similar methods,¹⁷ as might be expected from the relative success of the calculations previously reported.

Our interest in cooperative H-bonding in peptides has led us to study the vibrational properties of peptides and models of

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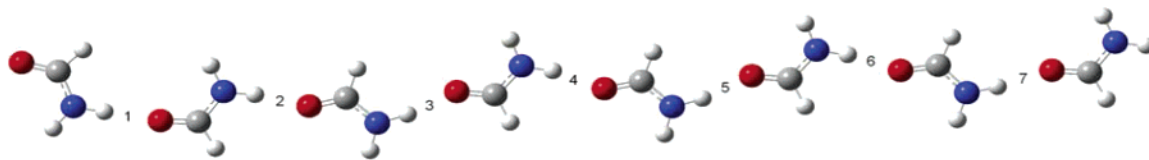


Figure 1. Completely optimized planar chain of eight formamides. The H-bond are numbered from left to right.

similar H-bonding systems.^{19,20} While previous theoretical studies have shown that the covalent peptide backbone structure proximate to individual H-bonds has little influence on the trans-H-bond coupling, no theoretical studies on the effect of proximate H-bonds have appeared, despite an experimental observation of such an effect.¹¹

Clearly, the relationship between structure and calculated trans-H-bond coupling constants must be firmly established if this new NMR technique is to prove useful in peptide structural studies.

In this paper we address several problems involving the relation between peptide structure and calculated trans-H-bond coupling constants. In particular, we report on the following: (a) the effect of proximate H-bonds on the individual trans-H-bond couplings in H-bonding formamide chains constructed with both optimized and identical repeating formamide units in both planar conformations and conformations taken from an alpha-helix; (b) the effects of changes in the local geometry of the H-bonds upon these couplings; and (c) effect of the errors inherent in the various approximations upon the accuracy of the structural information derived from the theoretical calculations.

Computational Details

All calculations were performed using the GAUSSIAN 03 suite of programs²¹ and the B3LYP functional. This method combines Becke's three-parameter functional,²² with the nonlocal correlation provided by the correlation functional of Lee, Yang, and Parr,²³ which has also been shown to be suitable for vibrational calculations.²⁴ We performed both (unrestricted) UB3LYP and (restricted) B3LYP calculations for several examples. As we found no differences in the results of the two methods, we used B3LYP alone for most of the calculations. All calculations used the D95(d,p) basis set which we have previously used in other studies of peptide and peptide-like H-bonds. We determined this basis set to be adequate for this purpose elsewhere,¹⁸ as it predicted trans-H-bond ^{13}C – ^{15}N three-bond scalar J -couplings that were similar to those predicted by other similar and larger basis sets. The geometries of the species calculated are either idealized or taken from previously optimized formamide chains²⁵ or alpha-helical structures,²⁶ as specified

below. The coupling constants were calculated using the "spin–spin" option in GAUSSIAN 03. This option calculates J 's including contributions from the spin–dipole (SD) and both diamagnetic (DSO) and paramagnetic (PSO) spin–orbit terms in addition to the FC term.^{27–30}

Results and Discussion

We first address the areas described in the Introduction individually, followed by a more complete discussion of the entire study.

Effect of Proximate H-Bonds. Several previous studies of ^{13}C – ^{15}N trans-H-bond couplings in peptides have relied upon the assumption that these couplings could be adequately modeled by performing calculations on formamide dimers whose structures were taken from those of individual amide H-bonding sites with the valences satisfied with H's or acetyl groups. On the other hand, at least one experimental study suggested that other proximate H-bonds significantly influence these couplings.¹¹ To investigate the influence of proximate H-bonds in H-bonding chains similar to those in peptides, we calculated the coupling constants for all the H-bonds in two different H-bonding chains each containing eight monomeric formamide units. In one, the geometry of the H-bonding chain, taken from a previous study,²⁵ was completely optimized with the constraint that it be planar (Figure 1). The other was optimized with the constraints that each formamide unit (and each H-bond) was kept identical and that the two H-bonding angles $\text{C}=\text{O}\cdots\text{H}$ and $\text{O}\cdots\text{H}-\text{N}$ be linear. Table 1 displays the calculated coupling constants for the completely optimized octamer, while Table 2 provides similar data for the constrained octamer. For each, the coupling constants were calculated from the octamer, itself, for each of the seven dimers that could be extracted by taking pairs of adjacent formamides, as well as, for each of the six trimers, five tetramers, four pentamers, three hexamers, and two heptamers that can be extracted in an analogous manner. For the octamer optimized with the constraint that each formamide unit and H-bond be kept equivalent, all of the dimers become identical as do each of the other component smaller aggregates that can be taken from the full structure. The data clearly show that adjacent H-bonds have an effect upon the coupling constants of each other. The effect upon a particular H-bond is stronger when the adjacent H-bond is added as a H-donor to it, rather than as an acceptor from it, in agreement with the experimental report.¹¹

Let us first consider the data for the chain optimized with the constraints, as these are independent of all geometric factors (such as H-bond distances and angles) other than the number of cooperatively interacting H-bonds. The data of Table 2 show that reasonable approximations to the correct calculated cou-

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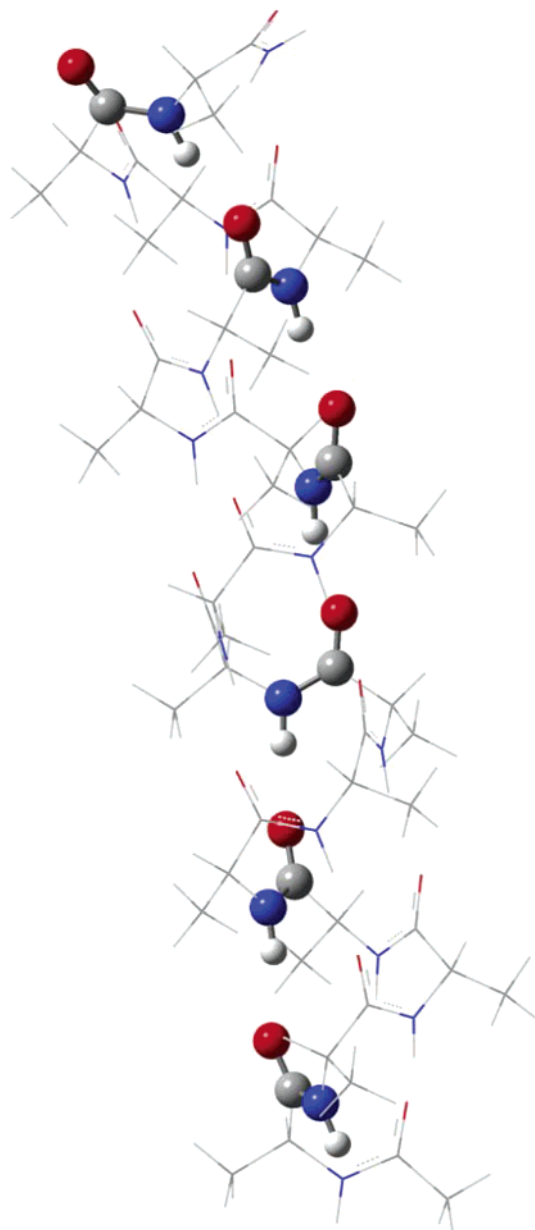


Figure 2. Acetyl(ala) $_{17}$ NH $_2$ with one of the three H-bonding chains emphasized.

13 (which use the O \cdots H distance) and 14 (which uses the N \cdots O distance) of his paper.¹² Equation 12 of this reference fits the geometric parameters to theoretical, while eqs 13 and 14 fit to experimental coupling constants. The agreement between these J 's and those for *dimers* of the present study is reasonably good for eq 12, despite the fact that Barfield used a different functional, different basis set, and only the FC term for his calculations. However, as one might expect, use of his equations does not reproduce the effect of the proximate H-bonds, which is included in the H-bonded chains. Thus the values of J predicted by eq 12 tend to be about 10% too low when compared to those we calculated using the H-bonding chains. The agreement of our data with those calculated using Barfield's eq 13 is clearly of lower quality. The differences between our data from the chains and these values is typically about 25%. However, the data calculated using eq 14, which is based on experimental N \cdots O distances, are in much better

agreement with our data for chains than for dimers. Nevertheless, there are significant errors for a few H-bonds. One should note that Barfield's correlations for eqs 12 and 14 are much higher than those for eq 13.

Effects of Local Geometry. Several previous papers report the effects of various changes in local geometry upon the magnitude of the trans-H-bond ^{13}C – ^{15}N three-bond scalar J -couplings (see discussion above). These reports have concentrated on the H-bonding distances (C \cdots N, O \cdots N or O \cdots H) and the valence and dihedral angles involving the H-bonds.^{5,12} The O \cdots H distance in an H-bond can be modified in several different ways without changing the C \cdots N distance. For example, increasing either the C=O or N–H distances while holding the C \cdots N distance constant will decrease the O \cdots H distance. Previous studies from our group have shown that amide C=O's tend to lengthen toward the center of H-bonding chains.^{20,26} To investigate the effects of these and other similar specific geometric variations in the local H-bonding geometries, we performed a series of calculations on formamide dimers and octamers where each molecule is equivalent. We began with the optimized structures with collinear C=O, N–H, and hydrogen bonds as discussed above. Various parameters were then modified to different fixed values to test the effects of variations in each of these parameters upon the calculated couplings. We specifically considered the effects of decreasing the O \cdots H distance by 0.01 Å in the dimer. This distance was reduced in three distinct ways: (1) by increasing the C=O bond length, (2) by increasing the N–H bond length, and (3) by decreasing the C \cdots N distance while the other distances and angles remain constant for each modification. The data in Table 4 clearly indicate that a change in the C=O bond length has the greatest effect of these three. An increase in the C=O distance of 0.01 Å causes the magnitude of the coupling to increase by 0.067 Hz (4.7%), an increase in N–H, 0.050 Hz (3.5%), and a decrease in C \cdots N, 0.046 Hz (3.1%). Each of these changes decreases the O \cdots H distance from 1.815 to 1.805 Å, in the example used here. That the C=O distance should have the largest effect of these three changes in distances is consistent with the extended π -delocalization that is thought to be operative in H-bond cooperativity for structures similar to those studied here.

The O \cdots H distance can also be reduced by changing either of the C=O \cdots H or O \cdots H–N angles. The effects of changing these parameters have been previously studied in a slightly different context.^{5,12} We considered reducing the O \cdots H distance by the same 0.01 Å by changing each of these angles while the C=O and N–H distances remained constant. In these cases the C \cdots N distances necessarily change. The data show that reducing the C=O \cdots H angle has the largest effect upon the coupling. Reducing this angle from 180° to 145° (the amount required to reduce the O \cdots H distance by 0.01 Å) lowers the calculated coupling by 0.542 Hz (37.7%), while lowering the O \cdots H–N angle by the same amount increases the coupling by 0.234 Hz (16.3%). Clearly changing the O \cdots H distance by modifying these angles has a greater effect than by modifying the distances considered above. It is particularly noteworthy that reducing the C=O \cdots H angle is the only modification that lowers the coupling while lowering the C \cdots N distance. As mentioned earlier, the more acute C=O \cdots H angles near the center of the completely optimized planar formamide octamer are responsible

Table 3. Comparison of trans-H-Bond ^{13}C – ^{15}N Three-Bond Scalar J -Couplings (Hz) for the 15 H-Bonds in Acetyl(ala) $_{17}$ NH $_2$ Using the Three H-Bonding Chains of the Alpha-Helix with Similar Calculations Using Only Formamide Dimers^a

geometric parameters					calculated ^{15}N – ^{13}C $^3\text{h}J$		using Barfield's eqs		
O..H	C=O	C=O..H	O..H–N	C...N	from dimer	from chain	eq 12	eq 13	eq 14
2.379	1.238	153.6	159.9	4.530	–0.101	–0.112	–0.150	–0.198	–0.141
1.995	1.234	152.0	166.3	4.153	–0.512	–0.554	–0.502	–0.427	–0.553
1.960	1.237	152.3	165.5	4.122	–0.562	–0.616	–0.562	–0.466	–0.628
1.950	1.237	150.8	164.1	4.105	–0.561	–0.608	–0.564	–0.480	–0.672
2.037	1.237	148.1	160.6	4.163	–0.421	–0.445	–0.411	–0.385	–0.519
2.010	1.233	152.5	167.6	4.170	–0.432	–0.460	–0.482	–0.413	–0.524
1.983	1.235	152.9	165.5	4.147	–0.528	–0.578	–0.528	–0.442	–0.584
1.968	1.237	151.3	164.5	4.124	–0.536	–0.589	–0.542	–0.460	–0.627
1.980	1.237	151.7	165.7	4.139	–0.520	–0.568	–0.524	–0.440	–0.577
2.031	1.236	141.0	152.8	4.086	–0.384	–0.405	–0.361	–0.395	–0.645
2.024	1.234	141.8	163.3	4.103	–0.362	–0.389	–0.378	–0.395	–0.536
1.955	1.236	152.6	165.5	4.120	–0.571	–0.625	–0.575	–0.474	–0.643
1.957	1.237	152.4	165.0	4.120	–0.575	–0.630	–0.569	–0.469	–0.637
1.973	1.237	149.8	163.8	4.120	–0.519	–0.566	–0.518	–0.457	–0.636
2.194	1.236	124.8	140.8	3.955	–0.121	–0.143	–0.133	–0.217	–0.330

^a Barfield's fits of J to local geometry using O...H or N...O (eqs 12, 13, and 14 of ref 12) are included for comparison (see text for discussion). The results are arranged with the H-bonds if the three chains are grouped together starting from the acetyl end of the structure.

Table 4. Effect of Variation of Different Geometric Parameters upon trans-H-Bond ^{13}C – ^{15}N Three-Bond Scalar J -Couplings (Hz) in a Model Formamide Dimer Optimized with a Linear H-Bond^a

N–H...O	C–O...H	C..N	C–O	N–H	O...H	J
177.8	175.9	4.156	1.225	1.016	1.919	–1.004
180.0	180.0	4.067	1.233	1.020	1.815	–1.436
180.0	180.0	4.067	1.243	1.020	<i>1.805</i>	–1.503
180.0	180.0	4.067	1.233	1.030	<i>1.805</i>	–1.486
180.0	180.0	4.057	1.233	1.020	<i>1.805</i>	–1.480
180.0	145.0	<i>3.900</i>	1.233	1.020	<i>1.805</i>	–0.894
145.0	180.0	<i>3.917</i>	1.233	1.020	<i>1.805</i>	–1.670
148.4	168.7	<i>3.959</i>	1.233	1.020	<i>1.805</i>	–1.534

^a The modified parameter for each entry is in boldface. For comparison, the completely optimized planar dimer is entered in italics.

Table 5. Trans-H-Bond ^{13}C – ^{15}N Three-Bond Scalar J -Couplings (Hz) for Each H-Bond in an Octamer as a Function of Geometric Variation^a

feature	optimization		linear optimization with perturbation ^b				
	complete	linear					
N–H...O	177.8	180.0	180.0	180.0	180.0	180.0	145.0
C=O...H	175.9	180.0	180.0	180.0	180.0	145.0	180.0
C..N	4.156	4.067	4.067	4.067	4.057	3.900	3.917
C=O	1.225	1.233	1.243	1.233	1.233	1.233	1.233
N–H	1.016	1.020	1.020	1.030	1.020	1.020	1.020
O...H	1.919	1.815	1.805	1.805	1.805	1.805	1.805

H-bond	^{13}C – ^{15}N trans-H-bond three-bond scalar $^3\text{h}J$ -couplings						
dimer	<i>–1.004</i>	<i>–1.436</i>	<i>–1.503</i>	<i>–1.486</i>	<i>–1.480</i>	<i>–0.894</i>	<i>–1.670</i>
1	–1.263	–1.479	–1.547	–1.531	–1.524	–0.890	–1.708
2	–1.244	–1.542	–1.612	–1.597	–1.587	–0.934	–1.773
3	–1.112	–1.554	–1.624	–1.609	–1.600	–0.942	–1.786
4	–1.075	–1.556	–1.627	–1.612	–1.602	–0.944	–1.789
5	–1.118	–1.555	–1.626	–1.611	–1.601	–0.944	–1.787
6	–1.243	–1.549	–1.620	–1.604	–1.595	–0.942	–1.780
7	–1.168	–1.519	–1.590	–1.573	–1.565	–0.955	–1.757

^a The values for the dimer with the corresponding geometry for each column are entered in italics. ^b Modified feature in boldface.

for the smaller couplings calculated near the center of this species despite the shorter H-bonds.

We applied the same changes in geometry used in the dimer to the octamer with regular repeating units. As can be seen from Table 5, the incremental changes in the calculated couplings at each H-bond for each of the three methods of shortening O...H are quite similar to those observed for the dimer. Thus,

Table 6. Percent Error Resulting from Neglect of the SD, DSO, and PSO Terms for Calculating the trans-H-Bond ^{13}C – ^{15}N Three-Bond Scalar J -Couplings in the Dimer and for Each H-Bond in the Regular Linear and the Completely Optimized Planar Octamers of Formamide Using B3LYP/D95(d,p)

H-bond	linear	planar
dimer	–6.3	–6.5
1	–5.4	–5.5
2	–4.8	–4.3
3	–4.7	–4.2
4	–4.6	–4.4
5	–4.6	–4.3
6	–4.7	–4.4
7	–5.2	–4.7

there seems to be little additional cooperative effect upon these changes in local geometry beyond those already discussed.

Use of the FC Term to Approximate J -Couplings. There are four major contributions to the calculated isotropic coupling constants: (1) the Fermi contact term (FC), (2) the spin-dipole interaction (SD), (3) the diamagnetic spin–orbit coupling (DSO), and (4) the paramagnetic spin–orbit coupling (PSO).³⁰ Previous calculations on the trans-H-bond couplings involved only calculations of the FC terms, as they are thought to be the major contributions to the total couplings. Since we have calculated these couplings with the inclusion of all four terms, we have revisited the appropriateness of approximating the couplings using only the FC terms.¹⁸ For the 15 basis sets considered, the FC term contributes from 90 to 95% of the value of J for this coupling (except for 6-31++G** where it contributes only 75%). For the D95** basis set used here, the FC term contributes 94% to the coupling. This value and the average contribution of 92% for all basis sets are in reasonable agreement with the 96% contribution of FC previously reported.^{14,33}

As can be seen from the data in Table 6, neglect of the three contributions to the individual H-bonds of the two formamide octamers to J other than FC generates errors that vary from –4.2 to –6.5%. Using only the FC term is slightly better for

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Table 7. Trans-H-Bond ^{13}C – ^1H Two-Bond Scalar $^2\text{h}J$ -Couplings for Each H-Bond in an Octamer as a Function of Geometric Variation^a

feature	optimization		linear optimization ^b				
	complete	linear					
N–H···O	177.8	180.0	180.0	180.0	180.0	180.0	145.0
C=O···H	175.9	180.0	180.0	180.0	180.0	180.0	180.0
C..N	4.156	4.067	4.067	4.067	4.057	3.900	3.917
C=O	1.225	1.233	1.243	1.233	1.233	1.233	1.233
N–H	1.016	1.020	1.020	1.030	1.020	1.20	1.020
O···H	1.919	1.815	1.805	1.805	1.805	1.805	1.805

H-bond	$^2\text{h}J^{13}\text{C}-^1\text{H}$	^{13}C – ^1H trans-H-bond scalar $^2\text{h}J$ -couplings						
		difference from $^3\text{h}J^{13}\text{C}-^{15}\text{N}$						
dimer		<i>–1.304</i>	<i>–0.132</i>	<i>–1.346</i>	<i>–1.369</i>	<i>–1.406</i>	<i>–1.060</i>	<i>–1.360</i>
1	–1.066	–1.245	–0.234	–1.283	–1.302	–1.336	–0.984	–1.361
2	–1.094	–1.189	–0.353	–1.223	–1.240	–1.270	–0.938	–1.323
3	–1.016	–1.177	–0.377	–1.210	–1.226	–1.255	–0.928	–1.315
4	–1.000	–1.174	–0.382	–1.207	–1.223	–1.252	–0.926	–1.313
5	–1.015	–1.176	–0.379	–1.209	–1.225	–1.254	–0.929	–1.314
6	–1.087	–1.185	–0.364	–1.220	–1.236	–1.266	–0.941	–1.321
7	–1.033	–1.231	–0.288	–1.269	–1.288	–1.321	–1.026	–1.360

^a The values for the dimer with the corresponding geometry for each column is entered in *italics*. The difference from the $^3\text{h}J^{13}\text{C}$ – ^{15}N couplings use the values for the linear optimized geometry from Table 5 for the C–N couplings. Distances are in angstroms, angles, in degrees, and J 's, in hertz. ^b Modified feature in boldface.

the more central H-bonds than those near the termini of the H-bonding chains. The J 's calculated from the FC terms, alone, will be relatively greater for the central H-bonds than if all four terms were used. Thus, calculations that approximate J using only the FC term will increase the apparent difference between the trans-H-bond ^{13}C – ^{15}N three-bond scalar J -couplings the H-bonds near the centers of H-bonding chains with respect to those near the ends, or calculated using only a dimer to approximate each H-bond.

Variation of trans-H-Bond ^{13}C – ^1H Two-Bond Scalar $^2\text{h}J$ -Couplings with Local Geometry and Proximate H-Bonds.

Since the trans-H-bond ^{13}C – ^1H two-bond scalar $^2\text{h}J$ -couplings can be calculated with reasonable accuracy using B3LYP and moderate basis sets,¹⁸ we consider whether they can provide structural information that compliments the more commonly measured ^{13}C – ^{15}N three-bond scalar $^3\text{h}J$ -couplings. An experimental report showed that these two different J 's were well correlated for ubiquitin.² Data for the ^{13}C – ^1H coupling constants as a function of local geometry in formamide dimer and formamide octamer are presented in Table 7. This table has the same general format as Table 5 (see earlier discussion for the details of the geometric variations) with the exception that a column indicating the difference between the ^{13}C – ^{15}N and ^{13}C – ^1H J 's has been added. Several differences from the data for the ^{13}C – ^{15}N couplings become immediately evident. Let us first consider the dimer. Whereas reducing the O···H distance by increasing the C=O bond length had the largest effect (of the three variations that maintain a linear H-bond) upon the ^{13}C – ^{15}N coupling, while increasing the C···N distance had the smallest, the order is reversed for the ^{13}C – ^1H couplings. On the other hand, changes in the two angles have effects that are qualitatively similar for the two J 's.

The data for the octamer show that proximate H-bonds reduce the ^{13}C – ^1H couplings, which is opposite of the effect upon the ^{13}C – ^{15}N couplings. The values of the differences between these two couplings for the linear systems (Table 7) indicate the fourth H-bond in the linear optimized octamer has the smallest ^{13}C – ^1H $^2\text{h}J$, but the largest difference from ^{13}C – ^{15}N $^3\text{h}J$. Thus, the differences between the two couplings can be an indication of

Table 8. Comparison of $^3\text{h}J^{13}\text{C}$ – ^{15}N and $^2\text{h}J^{13}\text{C}$ – ^1H trans-H-Bond Couplings for the Three H-bonding Chains Taken from the Optimized Alpha-Helical Structure of Acetyl(ala)₁₇NH₂

$J(\text{C}..N)$	$J(\text{C}..H)$	difference
	Chain 1	
–0.112	–0.095	–0.017
–0.554	–0.525	–0.029
–0.616	–0.590	–0.027
–0.608	–0.592	–0.016
–0.445	–0.428	–0.017
	Chain 2	
–0.460	–0.483	–0.022
–0.578	–0.543	–0.034
–0.589	–0.568	–0.021
–0.568	–0.551	–0.017
–0.405	–0.325	–0.079
	Chain 3	
–0.389	–0.389	–0.000
–0.625	–0.599	–0.026
–0.630	–0.598	–0.032
–0.566	–0.544	–0.022
–0.143	0.035	–0.179

the extent of cooperative interaction within an H-bonding chain. These data strongly suggest that the two coupling constants can provide complementary information, particularly where H-bonding chains are important structural features. The data of Table 8, which compare the two J 's for the three H-bonding chains of the alpha-helical structure previously discussed, further illustrate this point. Although the differences between the two kinds of J 's are not as large as those in the linear example of Table 7, they tend to be (1) greater for H-bonds nearer the center of the strand and (2) larger near the NH₂ than the acetyl end. The terminal H-bond on the NH₂ end has the largest difference, by far. However, this H-bond is atypical of an alpha-helix as both C=O···H and O···H–N angles are much more acute than the others (see Table 3).

Effects of Errors upon Correlations with Experimental Structures. Clearly, the level of accuracy required from the calculations will depend on the use to which they are put. The most common and significant application for calculating the trans-H-bond ^{13}C – ^{15}N three-bond scalar J -couplings is to aid

in the determination of the geometric parameters of important molecular structures significant in biological and biochemical studies. The same techniques could, in principle, be applied to other interesting H-bonding systems. Synthetic materials often use H-bonding interactions to achieve the desired self-assembled structures. Detailed structural knowledge of such materials would be an area to which the study of trans-H-bond ^{13}C – ^{15}N three-bond scalar J -couplings could be fruitfully applied.

The present study indicates that H-bond cooperativity influences the magnitudes of these couplings. Proximate H-bonds, particularly where the neighboring molecule acts as an H-bond donor, increase the magnitudes of the couplings. H-bonds in long chains have significantly larger couplings, particularly those nearest the centers of the chains. Thus the coupling for the central H-bond in a chain of eight identical formamides is 8.4% larger than that of an analogous dimer. For example, using a relationship derived by Barfield,¹² where J is proportional to $e^{-3.2(r_{\text{O}\cdots\text{H}} - 1.760)}$, we find an error of this magnitude corresponds to an error of about 0.02 Å in the estimate of O \cdots H. While this relationship was derived for a particular protein and we have seen that other local geometric parameters can influence the manner in which O \cdots H distance correlates with J , it provides a reasonable estimate of the order of magnitude for the error involved. If one determines the interaction enthalpy of such an H-bond using the quasi-linear relationship for H-bond enthalpies that we derived for H-bonding formamide chains,²⁵ this 0.02 Å error in O \cdots H corresponds to an error of about 1.4 kcal/mol in enthalpy. Such an error can be significant if one compares it to the estimated average H-bond enthalpy of a chain of eight formamides 9.9 kcal/mol, but even more so for H-bonds in an alpha-helix, where we have roughly estimated that acetyl-(ala)₁₇NH₂ has about 6.6 kcal/mol of strain per H-bond,²⁶ lowering the average H-bond energy to slightly more than 2 kcal/mol. One should note that such a helix has H-bonding chains of only six (rather than eight) H-bonds which would result in an error of 7.9% (rather than 8.4% noted above for a chain of eight formamides).

These cooperative effects will be important in those structures that have extensive H-bonding chains as structural features, such as alpha-helical peptides. They should be much less important in other structures that lack this feature. For example, many beta-sheet structures consist of only two antiparallel beta-strands, where there would be little or no contributions from H-bonding chains. The development of parallel methods, particularly those that can be applied to the same experimental system (such as the use of other trans-H-bond couplings) might prove useful for increasing the accuracy of NMR structural determinations of H-bonding systems.

Since intermolecular vibrations involving the H-bonding distances in gas-phase dimers such as (H₂O)₂ are rather anharmonic, the observed H-bonding distances generally are somewhat longer than predicted by the minima on the potential energy surfaces (PES). Although, calculation of the relevant anharmonic vibrations goes beyond the scope of this work, we can consider their likely qualitative effects upon the correlations of ^{13}C – ^{15}N J 's. The relevant vibrations become less anharmonic as the minima on the PES's become steeper and deeper. Thus, stronger H-bonds should be less anharmonic than weaker ones. In addition, the anharmonicities of H-bonds within a rigid framework should be less than those in one less rigid or in the

gas phase (where there is no exterior resistance to separation of the H-bonding monomers). Thus, H-bonds near the centers of helical or sheetlike structures might have less anharmonic H-bond vibrations than those near the ends of, or that do not participate in, such structures. As consideration of anharmonicities reduces the experimentally observed O \cdots O separation of 2.98 by about 0.03 Å for comparison to the PES of water dimer,^{34–36} the effect upon the peptide H-bonds must be somewhat less.

One must consider the effects of the dynamic geometries of molecules in solution and the effect upon the measured NMR spectra. Most of the correlations of theoretical and experimental coupling constants have used static geometries derived from crystal structures as the starting points for the MO calculations. A recent report of dynamical corrections to the calculations shows a large improvement in the correlation of calculated and measured J 's despite the fact that only the FC term was used in the calculation.¹³ Further consideration of dynamic effects, while extremely useful, would need a force field that includes the energetic cooperative effects of H-bonding chains that we have previously illustrated.^{25,26}

The importance of errors in the correspondence between ^{13}C – ^{15}N J 's and H-bond lengths will clearly depend on the use to which the data is put. For determining the H-bonding topology, the mere existence of the coupling would suffice. However, the assignment of a particular H-bond to its correct place in its proper structural motif (e.g., middle of an α -helix) would require a more precise determination of the H-bonding distance (and other local geometric parameters), as well as its position in an H-bonding chain (if any). The correlation of calculated J 's based upon experimental protein structures should be improved upon consideration of the local H-bonding environment. Calculation of ^{13}C – ^{15}N J 's in model structures in tandem with experimental determinations of them can become an important tool for peptide determination. For this level of interpretation, the relative H-bonding errors of about 0.02 Å can become significant. In principle, estimates of each of the H-bonding energies could be made from known relations between H-bonding distance and energies. As mentioned above, a 0.02 Å error in H-bond distance would introduce an unacceptable error in the corresponding H-bond energy.

In a very recent report (published after this paper was originally submitted), Cremer et al.³⁷ reported calculations on the ^{13}C – ^{15}N three-bond scalar 3J -couplings in ubiquitin, which has been repeatedly studied. They confirmed that the FC term dominates in these couplings (although not in others) and that the formamide dimer is a reasonable (dimer) approximation. Although they cited the experimental work of Juranic, they did not take these results into consideration in their analysis, which suggested that H-bonds in α -helices have smaller ^{13}C – ^{15}N three-bond scalar 3J -couplings than those in β -sheets with similar H-bonding distances. Cremer et al. also confirmed the validity of formamide dimers as reasonable approximations to dimers of more substituted amides as models for trans-H-bond coupling calculations. They did not consider models with more than one H-bond.

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Conclusions

The results reported here show that although DFT calculations can be usefully coupled with experimental observations of trans-H-bond ^{13}C – ^{15}N three-bond scalar 3hJ -couplings, proper caution must be used when interpreting the combined set of experimental and theoretical data to obtain structural information. In particular, H-bonding chains must be treated differently from isolated H-bonds. Within chains, two effects that are absent in simple isolated H-bonds become important. Enhanced coupling results when the H-bond whose J -coupling is being considered is part of such a chain. Entities that are H-bond donors have a larger effect upon the J -coupling of the proximate H-bond of interest than those that are H-bond acceptors, but both effects are significant. Furthermore, C=O bond distances tend to lengthen particularly near the center of long H-bonding chains. Since lengthening C=O bonds increases the magnitude of the J -couplings even when the C \cdots N distance is kept constant, H-bonds near the center of H-bonding chains should have larger J -couplings than those predicted from the C \cdots N distance alone even when the effects of neighboring H-bonds are considered. Both of these observations are in agreement with the experimental report of Juranic.¹¹

The magnitudes of the errors that can result will depend on the local secondary structure of the peptides or proteins studied.

Secondary structural motifs such as extended alpha-helices should require more attention to the specific detailed structures than others, such as beta-sheets, made up of only two strands. Thus, one must carefully consider the peptide structure beyond that of the individual, localized H-bonds to properly use the relationship between H-bond structure and trans-H-bond ^{13}C – ^{15}N three-bond scalar J -couplings.

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Supporting Information Available: Cartesian coordinates of the relevant structures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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