

# Dependence upon Basis Sets of trans Hydrogen-Bond $^{13}\text{C}$ – $^{15}\text{N}$ 3-Bond and Other Scalar J-Couplings in Amide Dimers Used as Peptide Models. A Density Functional Theory Study

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We report B3LYP DFT calculations of trans H-bond scalar  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond  $^3\text{hJ}$ -coupling constants using various basis sets on a model for peptide H-bonds formamide dimer. All four components to the J's (Fermi contact, FC, spin-dipole, SD, paramagnetic spin-orbit, PSO, and diamagnetic spin-orbit, DSO) have been calculated. These calculated couplings are reasonably independent of basis set except for small basis sets and are dominated by the FC terms. Other trans H-bond couplings (one-bond  $^{17}\text{O}$ – $^1\text{H}$   $^1\text{hJ}$ ; and the two-bond  $^{13}\text{C}$ – $^1\text{H}$  and  $^{15}\text{N}$ – $^{17}\text{O}$   $^2\text{hJ}$ 's) have also been calculated by the same methods. Their dependencies upon basis set, relative dominance by the FC terms, and potential utility for peptide structural studies are considered.

Reports of trans H-bond scalar  $^3\text{hJ}$  couplings in nucleic acids<sup>1</sup> and proteins,<sup>2–4</sup> have led to the measurement and use of these J-couplings for the study of the structures of peptides and proteins.<sup>5–13</sup> These couplings allow direct determination of H-bond topology and local structural details proximate individual H-bonds in complex peptides. Several recent reports correlate these couplings with H-bonding distances and angles involved.<sup>3,5,12</sup> Theoretical reports correlate calculated and measured couplings and predict couplings as a function of H-bond distance and related valence and torsional angles.<sup>12</sup> The theoretical studies have generally relied upon the importance of the fermi contact (FC) term to the overall coupling constant, as only this term has been calculated in most of these studies. Furthermore, 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.<sup>5</sup> 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.<sup>14</sup> A very recent report by Cremer et al. has generally confirmed these approximations for the  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar J-couplings, which were considered in the earlier publications but shown that they are not general.<sup>15</sup>

The many more reports of calculated NMR coupling constants on more traditional systems have been reviewed,<sup>16</sup> most recently by Contreras et al.<sup>17</sup> Coupling constants are among the most difficult molecular properties to calculate accurately. Increasing the complexity of the calculations by using very large basis sets does not necessarily lead to facile conversion to consistent or observed results.<sup>18</sup> Nevertheless, most theoretical calculations on trans H-bond couplings have (necessarily, due to their complexity) been performed with small to moderate basis sets. These results were *correlated* with experiment (they do not necessarily match experiment). Since moderately large peptides

can now be completely optimized<sup>19,20</sup> using DFT calculations with the ONIOM<sup>21–23</sup> methodology, we anticipate the ability to calculate trans H-bond J's on larger peptide fragments than previously reported. Juranic<sup>11</sup> has shown experimentally and we have theoretically<sup>24</sup> that the magnitudes of the  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar J-couplings in proteins and peptides depend on their positions in H-bonding chains. However, calculations of the  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar J-couplings in peptide containing even a few amino acid residues can only be accomplished with relative small to moderate basis sets. Thus, we believe it important to evaluate the performance of DFT calculations using such basis sets for the  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar J-couplings, as well as, other trans-H-bonding J-couplings that could potentially be used as analytic tools.

In this paper, we examine (a) the effects of basis set variation on these couplings; (b) the validity of using only the FC term to approximate the J's; and (c) the possibility that other trans H-bond coupling constants could provide additional structural information.

## Computational Details

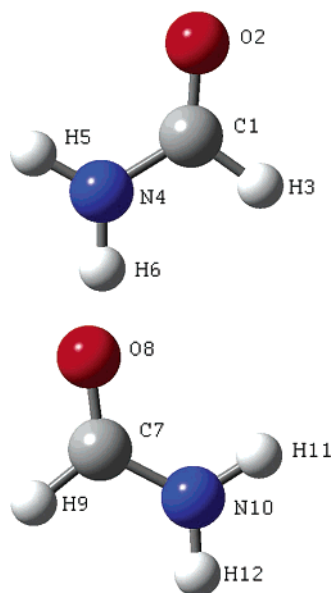
All calculations were performed using the Gaussian 03 suite of programs.<sup>25</sup> All calculations used the B3LYP functional. This method combines Becke's 3-parameter functional,<sup>26</sup> with the nonlocal correlation provided by the correlation functional of Lee, Yang, and Parr.<sup>27</sup> We performed both UB3LYP and 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. The coupling constants were calculated using the "spinspin" 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.<sup>28–30</sup>

## Results and Discussion

We performed all calculations on an idealized formamide dimer that was optimized with a linear H-bond at b3lyp/d95\*\* level of theory. The linear geometry was used as a reference for studying geometry dependence of the couplings, which has

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**Figure 1.** Geometry of the formamide dimer model used for the calculations.

**TABLE 1: Contributions from Fermi-Contact (FC), Spin-Dipole (SD), Paramagnetic and Diamagnetic Spin-Orbit (PSO and DSO) Terms and Complete trans H-Bond  $^{13}\text{C}$ – $^{15}\text{N}$  3-Bond Scalar J-Couplings (Hz) as a Function of Basis Set Calculated Using B3LYP**

| basis set         | FC     | SD     | PSO    | DSO   | J      | FC/J  |
|-------------------|--------|--------|--------|-------|--------|-------|
| 6-31G             | −0.745 | −0.027 | −0.043 | 0.017 | −0.798 | 0.934 |
| 6-31G*            | −1.044 | −0.026 | −0.042 | 0.017 | −1.095 | 0.954 |
| 6-31G**           | −1.048 | −0.026 | −0.042 | 0.017 | −1.099 | 0.954 |
| 6-31++G**         | −0.186 | −0.030 | −0.050 | 0.017 | −0.248 | 0.748 |
| 6-311G**          | −0.900 | −0.028 | −0.050 | 0.017 | −0.961 | 0.936 |
| 6-311++G**        | −0.853 | −0.033 | −0.062 | 0.017 | −0.931 | 0.916 |
| 6-311++G(2df,p)   | −0.876 | −0.034 | −0.064 | 0.017 | −0.957 | 0.915 |
| 6-311++G(3df,2pd) | −0.828 | −0.035 | −0.065 | 0.017 | −0.911 | 0.910 |
| D95               | −1.097 | −0.033 | −0.057 | 0.017 | −1.171 | 0.937 |
| D95**             | −0.938 | −0.031 | −0.052 | 0.017 | −1.004 | 0.935 |
| D95++**           | −0.947 | −0.033 | −0.054 | 0.017 | −1.016 | 0.932 |
| cc-pVDZ           | −0.680 | −0.027 | −0.046 | 0.017 | −0.735 | 0.924 |
| cc-pVTZ           | −0.855 | −0.031 | −0.056 | 0.017 | −0.924 | 0.925 |
| cc-pVQZ           | −0.894 | −0.035 | −0.063 | 0.017 | −0.974 | 0.918 |
| aug-cc-pVTZ       | −0.710 | −0.034 | −0.062 | 0.017 | −0.789 | 0.900 |

been reported elsewhere.<sup>24</sup> The numbering scheme is shown in Figure 1. In this structure the, both the  $\text{C}=\text{O}\cdots\text{H}$  and the  $\text{O}\cdots\text{H}-\text{N}$  angles were constrained to be linear. The  $\text{C}\cdots\text{N}$ ,  $\text{C}-\text{O}$ ,  $\text{N}-\text{H}$ , and  $\text{O}\cdots\text{H}$  distances are 4.156, 1.225, 1.016, and 1.919 Angstroms, respectively.

**Effect of Basis Sets upon the  $^{13}\text{C}$ – $^{15}\text{N}$  3-Bond Scalar J-Couplings.** As mentioned above, basis sets can have a significant effect upon the quality of calculated J-coupling constants, in general.<sup>18</sup> However, the effect of basis set variation specifically upon  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar J-couplings has not been previously reported. Since the use of extended basis sets for calculation of the couplings in large systems, such as peptide models, is precluded by the complexity of the calculations, we deemed it useful to determine the effects of basis set variation upon these couplings. To this end, we calculated the trans-bond coupling between  $^{15}\text{N}$  and  $^{13}\text{C}$  in a formamide dimer using 15 different basis sets taken from three different “families”: 6-31G, D95, and cc-pVXZ. We used several levels of basis set completeness within each family. The geometry of the dimer is taken from our earlier study of chains of H-bonding formamides.<sup>31</sup> The results presented in Table 1 are collected by “family”.

We present eight calculations performed with the 6-31G series of basis sets. The smallest basis set, 6-31G, predicts a J of  $-0.798$  Hz. Adding polarization functions on the heavy atoms increases the value to almost  $-1.1$  Hz. Polarization functions on the H’s have virtually no additional effect. Improving the main basis functions from 6-31G to 6-311G lowers the coupling by about 0.13 Hz (polarization functions are also used in the examples). Adding diffuse functions to the 6-31G(d,p) basis set causes the value of J to fall from  $-1.019$  to  $-0.248$  Hz, while adding the same diffuse functions to 6-311G(d,p) has only a small effect, lowering J by only 0.03 Hz. The 6-31++G(d,p) result, which seems anomalous, is reproducible. We have no compelling explanation of this result other than the known sensitivity of the calculated couplings to basis set variation. Extensions of the basis sets to 6-311++G(2df,p) and 6-311++G(3df,2pd) have small effects. The first increases the value of J from that of 6-311++G(d,p) to a value similar to that calculated for 6-311++G, and the second lowers the value of J to  $-0.911$ .

We present three calculations performed with the D95 and five with the cc-pVXZ series. The value of J calculated with D95 is  $-1.171$ , the largest magnitude for all of the basis sets studied. The magnitude decreases to  $-1.004$  for D95(d,p) and rises slightly to  $-1.016$  with D95++(d,p). With cc-pVDZ, the calculated value of J is  $-0.735$ , which is the smallest value of the entire study except for the seemingly anomalous value calculated with 6-31++G(d,p). This value increases in magnitude to  $-0.924$  and  $-0.974$  with cc-pVTZ and cc-pVQZ, respectively. When diffuse functions are added to cc-pVTZ to obtain cc-aug-pVTZ, the calculated value of J goes from  $-0.924$  to  $-0.789$  Hz. Thus, diffuse functions, which have little effect upon the trans H-bond couplings calculated with the 6-31 (except for the seemingly anomalous case already noted) and D95 series of basis sets, have a greater effect upon those calculated with cc-pVTZ. Computational limitations precluded the use of basis sets larger than aug-cc-pVTZ.

One hesitates to promulgate a firm conclusion on which basis set is best from the data at hand, particularly in the light of previous work on  $^2\text{J}$ -couplings.<sup>18</sup> Most of the current calculations made with reasonably good basis sets predict the trans H-bond J to be in the range of  $-0.96$  to  $-1.02$  Hz. Of the exceptions, two predict  $-0.91$  and  $-0.93$  and the other  $-0.79$ . Since D95-(d,p) predicts a value within this range ( $-1.004$ ), is simple enough to allow calculations on reasonably complex systems, and is the basis set that we have used extensively for previous calculations on peptides and other H-bonding systems, we have decided to continue its use in the further studies described that we present elsewhere.<sup>24</sup> We emphasize that, although this seems to us as a reasonably convenient choice based on the current data and our previous work, we do not mean to imply that this is the basis set of choice for all similar studies.

Of the three “families” of basis sets, the smallest basis sets of two (6-31G and cc-pVDZ) predict notably smaller J’s than the other (D95). Notably, the trans H-bond J is relatively insensitive (compared to the other series) to basis set augmentation within the D95 series of basis sets. Since the largest contributing term to this coupling is FC, the fact that D95 devotes more primitive Gaussians to the 1s orbital (which is the orbital with the density at the nucleus) might be significant. Additional studies that correlate the number and quality of primitive Gaussians in the 1s orbital with the calculated coupling constants and FC’s would be necessary to confirm this suggestion. These are beyond the scope of this paper.

One can definitely conclude that 6-31++G(d,p) should be avoided for calculations of trans H-bond  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar

**TABLE 2: Contributions from Fermi-Contact (FC), Spin-Dipole (SD), Paramagnetic and Diamagnetic Spin-Orbit (PSO and DSO) Terms and Complete trans H-Bond  $^{17}\text{O}-^{1}\text{H}$  1-Bond Scalar  $^1\text{hJ}$ -Couplings (Hz) as a Function of Basis Set Calculated Using B3LYP**

| basis set         | FC    | SD     | PSO   | DSO    | J     | FC/J  |
|-------------------|-------|--------|-------|--------|-------|-------|
| 6-31G             | 6.005 | -0.234 | 0.589 | -0.536 | 5.824 | 1.031 |
| 6-31G*            | 6.275 | -0.229 | 0.815 | -0.566 | 6.295 | 0.997 |
| 6-31G**           | 6.290 | -0.224 | 0.971 | -0.577 | 6.460 | 0.974 |
| 6-31++G**         | 5.637 | -0.222 | 0.959 | -0.577 | 5.797 | 0.972 |
| 6-311G**          | 6.580 | -0.249 | 1.115 | -0.591 | 6.856 | 0.960 |
| 6-311++G**        | 6.210 | -0.244 | 1.163 | -0.591 | 6.538 | 0.950 |
| 6-311++G(2df,p)   | 6.273 | -0.244 | 1.150 | -0.593 | 6.585 | 0.953 |
| 6-311++G(3df,2pd) | 6.145 | -0.247 | 1.215 | -0.598 | 6.515 | 0.943 |
| D95               | 6.893 | -0.225 | 0.607 | -0.534 | 6.742 | 1.022 |
| D95**             | 6.689 | -0.221 | 1.036 | -0.579 | 6.924 | 0.966 |
| D95++**           | 6.621 | -0.222 | 0.983 | -0.580 | 6.802 | 0.973 |
| cc-pVDZ           | 5.572 | -0.223 | 1.039 | -0.584 | 5.804 | 0.960 |
| cc-pVTZ           | 6.055 | -0.236 | 1.121 | -0.597 | 6.343 | 0.955 |
| cc-pVQZ           | 6.681 | -0.242 | 1.255 | -0.602 | 7.092 | 0.942 |
| aug-cc-pVTZ       | 6.078 | -0.239 | 1.183 | -0.597 | 6.426 | 0.946 |

**TABLE 3: Contributions from Fermi-Contact (FC), Spin-Dipole (SD), Paramagnetic and Diamagnetic Spin-Orbit (PSO and DSO) Terms and Complete trans H-Bond  $^{13}\text{C}-^{1}\text{H}$  2-Bond Scalar  $^2\text{hJ}$ -Couplings (Hz) as a Function of Basis Set Calculated Using B3LYP**

| basis set         | FC     | SD    | PSO   | DSO    | J      | FC/J  |
|-------------------|--------|-------|-------|--------|--------|-------|
| 6-31G             | -0.890 | 0.108 | 0.379 | -0.204 | -0.607 | 1.466 |
| 6-31G*            | -0.953 | 0.114 | 0.381 | -0.210 | -0.668 | 1.427 |
| 6-31G**           | -0.928 | 0.117 | 0.330 | -0.203 | -0.684 | 1.357 |
| 6-31++G**         | -1.058 | 0.108 | 0.289 | -0.201 | -0.861 | 1.229 |
| 6-311G**          | -1.143 | 0.107 | 0.417 | -0.203 | -0.821 | 1.392 |
| 6-311++G**        | -1.146 | 0.109 | 0.437 | -0.202 | -0.802 | 1.429 |
| 6-311++G(2df,p)   | -1.137 | 0.127 | 0.483 | -0.204 | -0.731 | 1.555 |
| 6-311++G(3df,2pd) | -1.092 | 0.136 | 0.498 | -0.201 | -0.659 | 1.657 |
| D95               | -1.289 | 0.115 | 0.468 | -0.205 | -0.911 | 1.415 |
| D95**             | -1.143 | 0.122 | 0.370 | -0.203 | -0.853 | 1.340 |
| D95++**           | -1.201 | 0.118 | 0.339 | -0.202 | -0.946 | 1.270 |
| cc-pVDZ           | -0.745 | 0.124 | 0.387 | -0.203 | -0.437 | 1.705 |
| cc-pVTZ           | -1.092 | 0.129 | 0.458 | -0.202 | -0.708 | 1.542 |
| cc-pVQZ           | -1.214 | 0.137 | 0.487 | -0.201 | -0.790 | 1.537 |
| aug-cc-pVTZ       | -1.091 | 0.135 | 0.456 | -0.202 | -0.702 | 1.554 |

J-couplings. This basis set seems to have similar problems for the calculation of some (but not all) of the other trans H-bond coupling constants (see further discussion below).

**Other trans H-Bond J-Couplings.** Other trans H-bond couplings in addition to the trans  $^{13}\text{C}-^{15}\text{N}$  3-bond scalar  $^3\text{hJ}$ -couplings can be calculated and be measured, at least in principle. The most obvious couplings that might be considered include the one-bond  $^{17}\text{O}-^{1}\text{H}$   $^1\text{hJ}$ ; and the two-bond  $^{13}\text{C}-^{1}\text{H}$  and  $^{15}\text{N}-^{17}\text{O}$   $^2\text{hJ}$ 's. Although we know of only one report<sup>2</sup> of the use of one of these J's for structural determinations of peptides or proteins, we present data for these J's for the optimized formamide as calculated using the 15 basis sets that we used for the trans  $^{13}\text{C}-^{15}\text{N}$  3-bond scalar  $^3\text{hJ}$ -couplings since these data is already available from the results of latter calculations. The data for each of these J's are presented in Tables 2–5, each of which has the same format as Table 1.

**$^{17}\text{O}-^{1}\text{H}$  1-Bond Scalar  $^1\text{hJ}$ -Couplings.** The trans H-bond  $^{17}\text{O}-^{1}\text{H}$  1-bond scalar  $^1\text{hJ}$ -couplings are about an order of magnitude greater than the trans H-bond  $^{13}\text{C}-^{15}\text{N}$  3-bond scalar  $^3\text{hJ}$ -couplings that are principally used for studying peptide structures. The 6-31++G(d,p) basis set again predicts a smaller coupling than the others due to the FC term. As seen from Table 2, the FC terms dominate. However, the total J's are slightly larger than FC term except for in the small basis set 6-31G. The differences between the FC contributions and the total J's principally due to the large variation in the PSO term with basis

**TABLE 4: Contributions from Fermi-Contact (FC), Spin-Dipole (SD), Paramagnetic and Diamagnetic Spin-Orbit (PSO and DSO) Terms and Complete trans H-Bond  $^{15}\text{N}-^{17}\text{O}$  2-Bond Scalar  $^2\text{hJ}$ -Couplings (Hz) as a Function of Basis Set Calculated Using B3LYP**

| basis set         | FC    | SD    | PSO    | DSO   | J     | FC/J  |
|-------------------|-------|-------|--------|-------|-------|-------|
| 6-31G             | 5.152 | 0.030 | -0.039 | 0.013 | 5.156 | 0.999 |
| 6-31G*            | 6.206 | 0.032 | -0.051 | 0.015 | 6.202 | 1.001 |
| 6-31G**           | 6.240 | 0.032 | -0.050 | 0.015 | 6.236 | 1.001 |
| 6-31++G**         | 3.940 | 0.028 | -0.069 | 0.014 | 3.913 | 1.007 |
| 6-311G**          | 5.261 | 0.038 | -0.046 | 0.015 | 5.268 | 0.999 |
| 6-311++G**        | 5.155 | 0.031 | -0.076 | 0.015 | 5.126 | 1.006 |
| 6-311++G(2df,p)   | 5.108 | 0.032 | -0.076 | 0.015 | 5.079 | 1.006 |
| 6-311++G(3df,2pd) | 5.209 | 0.033 | -0.080 | 0.015 | 5.177 | 1.006 |
| D95               | 6.246 | 0.028 | -0.055 | 0.013 | 6.232 | 1.002 |
| D95**             | 5.869 | 0.029 | -0.071 | 0.015 | 5.843 | 1.004 |
| D95++**           | 5.732 | 0.029 | -0.072 | 0.015 | 5.704 | 1.005 |
| cc-pVDZ           | 5.070 | 0.034 | -0.054 | 0.015 | 5.064 | 1.001 |
| cc-pVTZ           | 5.021 | 0.035 | -0.059 | 0.015 | 5.012 | 1.002 |
| cc-pVQZ           | 5.382 | 0.034 | -0.077 | 0.016 | 5.354 | 1.005 |
| aug-cc-pVTZ       | 4.785 | 0.031 | -0.079 | 0.015 | 4.752 | 1.007 |

**TABLE 5: Contributions from Fermi-Contact (FC), Spin-Dipole (SD), Paramagnetic and Diamagnetic Spin-Orbit (PSO and DSO) Terms and Complete trans H-Bond  $^1\text{H}-^{15}\text{N}$  3-Bond Scalar  $^3\text{hJ}$ -Couplings**

| basis set         | FC     | SD    | PSO   | DSO   | J     | FC/J   |
|-------------------|--------|-------|-------|-------|-------|--------|
| 6-31G             | -0.029 | 0.046 | 0.063 | 0.019 | 0.099 | -0.295 |
| 6-31G*            | -0.029 | 0.042 | 0.041 | 0.019 | 0.073 | -0.401 |
| 6-31G**           | -0.031 | 0.041 | 0.055 | 0.017 | 0.082 | -0.374 |
| 6-31++G**         | -0.028 | 0.039 | 0.052 | 0.017 | 0.080 | -0.349 |
| 6-311G**          | -0.037 | 0.046 | 0.070 | 0.017 | 0.096 | -0.387 |
| 6-311++G**        | -0.034 | 0.042 | 0.063 | 0.017 | 0.087 | -0.391 |
| 6-311++G(2df,p)   | -0.035 | 0.042 | 0.051 | 0.017 | 0.074 | -0.476 |
| 6-311++G(3df,2pd) | -0.030 | 0.042 | 0.047 | 0.017 | 0.075 | -0.397 |
| D95               | -0.031 | 0.048 | 0.063 | 0.019 | 0.100 | -0.306 |
| D95**             | -0.032 | 0.042 | 0.060 | 0.017 | 0.088 | -0.361 |
| D95++**           | -0.036 | 0.041 | 0.059 | 0.017 | 0.082 | -0.438 |
| cc-pVDZ           | -0.030 | 0.043 | 0.061 | 0.017 | 0.091 | -0.328 |
| cc-pVTZ           | -0.030 | 0.044 | 0.059 | 0.017 | 0.090 | -0.332 |
| cc-pVQZ           | -0.030 | 0.043 | 0.052 | 0.017 | 0.082 | -0.369 |
| aug-cc-pVTZ       | -0.029 | 0.041 | 0.050 | 0.017 | 0.079 | -0.362 |

set. The large magnitude of FC terms render this variation relatively unimportant. As in the case of the  $^{13}\text{C}-^{15}\text{N}$  3-bond scalar J-couplings, the 6-31++G(d,p) basis set predicts the FC term to be anomalously low by about 0.6 Hz compared to the other basis sets and by about 0.7–0.8 Hz compared to the  $^{13}\text{C}-^{15}\text{N}$  3-bond scalar J-couplings. However, The larger magnitudes of the FC terms somewhat mask this apparent anomaly. These J's could be useful for determination of peptide structures by correlation of experimental and calculated values.

**$^{13}\text{C}-^{1}\text{H}$  2-Bond Scalar  $^2\text{hJ}$ -Couplings.** The trans H-bond  $^{13}\text{C}-^{1}\text{H}$  2-bond scalar  $^2\text{hJ}$ -couplings are similar in magnitude to the  $^{13}\text{C}-^{15}\text{N}$  3-bond couplings (see Table 3). Although the FC term makes the greatest contribution to the total J, the considerable contribution of the PSO term, which has a fairly large basis set dependence, precludes reliance on the FC term, alone. Unlike for the three other coupling constants studied (see tables 1, 2 and 4), the 6-31++G(d,p) basis set appears to predict an FC term that is consistent with those derived using the other basis sets. The calculated J's are greater for the D95 “family” than the others. They also vary more with basis set (and without clear trends) within each “family” than the  $^{13}\text{C}-^{15}\text{N}$  J's. If these J's are to be used for structure determination by correlation of calculated and experiment values, calculations at a higher level than we present here will be necessary. In all likelihood, these will be impractical for all but the smallest H-bond models. Thus, they will not be useful for calculation on moderately sized peptides.



**$^{15}\text{N}$ – $^{17}\text{O}$  2-Bond Scalar  $^{2h}\text{J}$ -Couplings.** The trans H-bond  $^{15}\text{N}$ – $^{17}\text{O}$  2-bond scalar  $^{2h}\text{J}$ -couplings are only slightly smaller in magnitude than the  $^{17}\text{O}$ – $^1\text{H}$  couplings (see Table 4). However, the effects of SD, PSO, and DSO are all small, virtually invariant with change in basis set and tend to cancel, with the PSO term being equal in magnitude and of opposite sign to the sum of the other two. Thus, the dominant FC term can describe the J's extremely well. However, there seem to be significant differences in the results predicted using different basis sets. Furthermore, there are no obvious trends with increasing basis set quality. For example, the J for the 6-31 series first increases, and then decreases with increasing bases, whereas the D95 and cc-pVXZ series predict J's that increase and decrease, respectively, as the basis sets grow in size. Here, as in the trans  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar  $^{3h}\text{J}$ -couplings, the 6-31++G(d,p) basis set predicts a value for the FC term (hence the total J) that differs significantly from that calculated using the other basis sets. Once again, utilization of the J's for structure determination by correlation of experimental and theoretical J's will not likely be useful for all but the smallest of H-bonding models.

**$^1\text{H}$ – $^{15}\text{N}$  3-Bond Scalar  $^{3h}\text{J}$ -Couplings.** The  $^1\text{H}$ – $^{15}\text{N}$  3-bond scalar  $^{3h}\text{J}$ -couplings (Table 5) are relatively insensitive to basis set variation. However, their uniformly small predicted magnitude of only about 0.1 Hz make them extremely poor candidates for experimental measurement. This is primarily due to the very small FC terms which not only are only from 30 to 43% of the total J's, but are of opposite sign. Thus, the other three terms (all of which are positive) are sufficiently larger than the FC terms to reverse the sign of the J's. Again, these J's are unlikely to be useful for structure determination of peptides from correlation of experimental and theoretical J's. However, the problem here lies principally with the small magnitude of the J's, rather than the level of calculation needed.

**Dimer trans H-Bond J's Not Relevant to Peptides.** Other trans H-bond couplings can be calculated for the dimer that will not be useful for peptide analysis. All are  $^1\text{H}$ – $^1\text{H}$  couplings: the  $^{3h}\text{J}$  between  $\text{H}_6$  and  $\text{H}_9$  and the  $^{4h}\text{J}$ 's between  $\text{H}_6$  and  $\text{H}_{11}$  and  $\text{H}_{12}$ . The first two of these J's are between  $\text{H}_6$  and H's that would normally be C's in a peptide chain. The third is between  $\text{H}_6$  and the H on the other formamide which would often form an H-bond in a peptide. We include the effects of basis set variation upon these couplings for completeness and to illustrate the errors that one can encounter when calculating J's of this type, as they provide excellent examples of the errors that might be anticipated.

**$^1\text{H}_6$ – $^1\text{H}_9$  3-Bond Scalar  $^{3h}\text{J}$ -Couplings.** These J's are dominated primarily by the PSO and DSO each of which are at least an order of magnitude greater than the FC and SD contributions (see Table 6). However, they are of opposite sign. Although the DSO term remains relatively constant at approximately  $-0.6$  Hz, the PSO term, which is quite sensitive to the variation of basis set, tends to become more positive with improvement of the basis. As a result, the  $^1\text{H}_6$ – $^1\text{H}_9$  3-bond scalar  $^{3h}\text{J}$ -couplings calculated with small basis sets seem to be within the range that might be useful for structure determination. However, improvement of the bases suggests that these J's might become vanishing small at a limiting basis. Of course, these H's do not exist in peptides as a skeletal carbon takes the position of  $\text{H}_9$  in the dimer model. The corresponding  $^1\text{H}$ – $^{13}\text{C}$  3-bond scalar  $^{3h}\text{J}$ -couplings could merit further investigation.

**$^1\text{H}_6$ – $^1\text{H}_{11}$  3-Bond Scalar  $^{4h}\text{J}$ -Couplings.** The  $^1\text{H}_6$ – $^1\text{H}_{11}$  3-bond scalar  $^{4h}\text{J}$ -couplings presented in Table 7 are again dominated by the PSO and DSO terms rather than the FC's.

**TABLE 6: Contributions from Fermi-Contact (FC), Spin-Dipole (SD), Paramagnetic and Diamagnetic Spin-Orbit (PSO and DSO) Terms and Complete trans H-Bond  $^1\text{H}$ – $^1\text{H}$  3-Bond Scalar  $^{3h}\text{J}$ -Couplings (Hz) as a Function of Basis Set Calculated Using B3LYP**

| basis set         | FC     | SD     | PSO   | DSO    | J      | FC/J  |
|-------------------|--------|--------|-------|--------|--------|-------|
| 6-31G             | −0.033 | −0.004 | 0.164 | −0.593 | −0.466 | 0.071 |
| 6-31G*            | −0.038 | 0.005  | 0.455 | −0.591 | −0.169 | 0.228 |
| 6-31G**           | −0.038 | 0.005  | 0.394 | −0.582 | −0.220 | 0.173 |
| 6-31++G**         | −0.033 | 0.006  | 0.404 | −0.580 | −0.203 | 0.163 |
| 6-311G**          | −0.038 | 0.005  | 0.465 | −0.583 | −0.152 | 0.249 |
| 6-311++G**        | −0.029 | 0.005  | 0.468 | −0.582 | −0.138 | 0.211 |
| 6-311++G(2df,p)   | −0.032 | 0.007  | 0.540 | −0.581 | −0.066 | 0.478 |
| 6-311++G(3df,2pd) | −0.032 | 0.007  | 0.559 | −0.579 | −0.045 | 0.719 |
| D95               | −0.030 | −0.006 | 0.113 | −0.593 | −0.517 | 0.057 |
| D95**             | −0.033 | 0.002  | 0.358 | −0.584 | −0.257 | 0.129 |
| D95+***           | −0.028 | 0.006  | 0.371 | −0.582 | −0.233 | 0.120 |
| cc-pVDZ           | −0.035 | 0.006  | 0.505 | −0.580 | −0.103 | 0.338 |
| cc-pVTZ           | −0.036 | 0.003  | 0.342 | −0.582 | −0.273 | 0.130 |
| cc-pVQZ           | −0.042 | 0.007  | 0.591 | −0.579 | −0.022 | 1.870 |
| aug-cc-pVTZ       | −0.031 | 0.007  | 0.529 | −0.579 | −0.073 | 0.420 |

**TABLE 7: Contributions from Fermi-Contact (FC), Spin-Dipole (SD), Paramagnetic and Diamagnetic Spin-Orbit (PSO and DSO) Terms and Complete trans H-Bond  $^1\text{H}_6$ – $^1\text{H}_{11}$  3-Bond Scalar  $^{3h}\text{J}$ -Couplings (Hz) as a Function of Basis Set Calculated Using B3LYP**

| basis set         | FC     | SD     | PSO    | DSO   | J      | FC/J   |
|-------------------|--------|--------|--------|-------|--------|--------|
| 6-31G             | −0.024 | −0.022 | −0.227 | 0.455 | 0.182  | −0.134 |
| 6-31G*            | −0.021 | −0.019 | −0.298 | 0.464 | 0.126  | −0.169 |
| 6-31G**           | −0.021 | −0.020 | −0.390 | 0.478 | 0.047  | −0.459 |
| 6-31++G**         | −0.019 | −0.017 | −0.389 | 0.478 | 0.053  | −0.358 |
| 6-311G**          | −0.022 | −0.019 | −0.475 | 0.474 | −0.042 | 0.522  |
| 6-311++G**        | −0.021 | −0.017 | −0.470 | 0.473 | −0.034 | 0.611  |
| 6-311++G(2df,p)   | −0.019 | −0.017 | −0.441 | 0.474 | −0.003 | 6.121  |
| 6-311++G(3df,2pd) | −0.019 | −0.017 | −0.446 | 0.480 | −0.002 | 11.136 |
| D95               | −0.025 | −0.020 | −0.197 | 0.453 | 0.212  | −0.120 |
| D95**             | −0.023 | −0.018 | −0.380 | 0.475 | 0.054  | −0.420 |
| D95+***           | −0.021 | −0.017 | −0.387 | 0.475 | 0.050  | −0.411 |
| cc-pVDZ           | −0.020 | −0.020 | −0.418 | 0.475 | 0.018  | −1.137 |
| cc-pVTZ           | −0.020 | −0.018 | −0.454 | 0.480 | −0.012 | 1.638  |
| cc-pVQZ           | −0.020 | −0.017 | −0.483 | 0.481 | −0.038 | 0.519  |
| aug-cc-pVTZ       | −0.020 | −0.016 | −0.439 | 0.479 | 0.005  | −3.952 |

Again the PSO and DSO terms are of opposite sign and tend to cancel. The DSO remains at slightly less than 0.5 Hz (positive), whereas the PSO (negative) varies in magnitude from about 0.2 to 0.5 Hz. For all but the smallest basis sets, J is less than 0.05 Hz with variation between positive and negative sign. This hydrogen could be considered to be replaced by a carbon in some peptide conformations, such as the  $\alpha$ -helix, where the  $\text{O}=\text{C}-\text{N}-\text{C}$  angle would be less than 90 degrees.

**$^1\text{H}_6$ – $^1\text{H}_{12}$  3-Bond Scalar  $^{4h}\text{J}$ -Couplings.** These J's (Table 8) behave somewhat similar to the other  $^1\text{H}$ – $^1\text{H}$  couplings discussed above except that the DSO's, which are relatively constant at  $-0.89$  Hz are not completely canceled by the positive PSO's which vary from about 0.2 to 0.8 Hz. The larger basis sets predict the large DSO's, thus the smallest total J's. All of the J's remain negative. Only one is less than 0.1 Hz in magnitude. These H's would be replaced by C's in some peptide conformations, notably  $\beta$ -sheets or  $\beta$ -strands.

The  $^1\text{H}_6$ – $^1\text{H}_{12}$  3-bond scalar  $^{4h}\text{J}$ -couplings involve hydrogens that may form other peptide H-bonds (in  $\alpha$ -helical or  $\beta$ -sheet conformations) or they may H-bond to solvent. The current data do not allow us to assess the effects that these H-bonds might have upon their J's.

**Importance of the Fermi Contact (FC) Term.** The data presented above indicate that the FC term can usefully approximate certain of the trans-H-bond J's that we have considered, but not all. As previously noted, the FC term has been used to approximate  $^{13}\text{C}$ – $^{15}\text{N}$   $^{3h}\text{J}$ 's with success. This term has been reported to account for 96% the coupling in dimers of

**TABLE 8: Contributions from Fermi-Contact (FC), Spin-Dipole (SD), Paramagnetic and Diamagnetic Spin-Orbit (PSO and DSO) Terms and Complete trans H-Bond  $^1\text{H}_6$ – $^1\text{H}_{12}$  3-Bond Scalar  $^3\text{hJ}$ -Couplings (Hz) as a Function of Basis Set Calculated Using B3LYP**

| basis set         | FC     | SD    | PSO   | DSO    | J      | FC/J  |
|-------------------|--------|-------|-------|--------|--------|-------|
| 6-31G             | −0.033 | 0.049 | 0.195 | −0.891 | −0.680 | 0.048 |
| 6-31G*            | −0.028 | 0.047 | 0.549 | −0.892 | −0.323 | 0.085 |
| 6-31G**           | −0.027 | 0.046 | 0.537 | −0.892 | −0.337 | 0.082 |
| 6-31++G**         | −0.024 | 0.042 | 0.554 | −0.891 | −0.318 | 0.075 |
| 6-311G**          | −0.029 | 0.045 | 0.670 | −0.891 | −0.205 | 0.139 |
| 6-311++G**        | −0.026 | 0.042 | 0.679 | −0.890 | −0.195 | 0.132 |
| 6-311++G(2df,p)   | −0.025 | 0.040 | 0.743 | −0.891 | −0.133 | 0.190 |
| 6-311++G(3df,2pd) | −0.025 | 0.040 | 0.769 | −0.891 | −0.107 | 0.235 |
| D95               | −0.032 | 0.048 | 0.176 | −0.890 | −0.698 | 0.046 |
| D95**             | −0.026 | 0.045 | 0.519 | −0.891 | −0.354 | 0.074 |
| D95++**           | −0.026 | 0.043 | 0.533 | −0.890 | −0.340 | 0.077 |
| cc-pVDZ           | −0.026 | 0.047 | 0.525 | −0.892 | −0.345 | 0.075 |
| cc-pVTZ           | −0.025 | 0.042 | 0.718 | −0.892 | −0.157 | 0.161 |
| cc-pVQZ           | −0.027 | 0.041 | 0.823 | −0.892 | −0.054 | 0.500 |
| aug-cc-pVTZ       | −0.025 | 0.040 | 0.743 | −0.891 | −0.134 | 0.187 |

*N*-methylacetamide.<sup>14</sup> The data in table agree reasonably well, with FC/J varying from 0.90 to 0.95 (not counting the anomalous result for 6-31++G(d,p)). However, the ratio tends to be lower with the larger basis sets. The variation of the ratio results from generally increasing magnitudes of the SD and PSO terms and somewhat decreasing FC contributions as the basis sets improve. The DSO term does not vary at all.

For the other trans H-bond J's discussed above, the FC term dominates the  $^{17}\text{O}$ – $^1\text{H}$  1-bond scalar  $^1\text{hJ}$ -couplings (Table 2) and the  $^{15}\text{N}$ – $^{17}\text{O}$  2-bond scalar  $^2\text{hJ}$ -couplings (Table 4), but not the  $^{13}\text{C}$ – $^1\text{H}$  2-bond scalar  $^2\text{hJ}$ -couplings (Table 3) the  $^1\text{H}$ – $^{15}\text{N}$  3-bond scalar  $^3\text{hJ}$ -couplings (Table 5) and the three different  $^1\text{H}$ – $^1\text{H}$   $^3\text{hJ}$  and  $^4\text{hJ}$ 's (Tables 6–8). In fact the relevant FC terms are nearly equivalent to the  $^{17}\text{O}$ – $^1\text{H}$  1-bond scalar  $^1\text{hJ}$ -couplings and the  $^{15}\text{N}$ – $^{17}\text{O}$  2-bond scalar  $^2\text{hJ}$ -couplings due to the cancellation of the contributions of the other three terms. The FC terms are generally slightly smaller in magnitude than the  $^{17}\text{O}$ – $^1\text{H}$  1-bond scalar  $^1\text{hJ}$ -couplings, while slightly larger than the  $^{15}\text{N}$ – $^{17}\text{O}$  2-bond scalar  $^2\text{hJ}$ -couplings. However, the FC terms are considerably larger than the  $^{13}\text{C}$ – $^1\text{H}$  2-bond scalar  $^2\text{hJ}$ -couplings. As they vary from 1.271 to 1.705 times the J's (again not considering 6-31++G(d,p)), the FC terms would not provide reliable estimates of the  $^{13}\text{C}$ – $^1\text{H}$  2-bond scalar  $^2\text{hJ}$ -couplings. Among the four J's that involve the H-bonding hydrogen ( $\text{H}_6$ ), the FC terms dominates only the  $^{17}\text{O}$ – $^1\text{H}$  1-bond scalar  $^1\text{hJ}$ -couplings.

## General Discussion

Juranic has shown the trans H-bond  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar J-couplings to be dependent upon the position of the H-bond within a H-bonding chain.<sup>10,11</sup> We have confirmed and further elucidated this dependence with DFT calculations.<sup>24</sup> Thus, the local geometry about the H-bonds cannot be unambiguously determined simply from the  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar J-couplings. Although the position of the H-bond in question within an H-bonding chain will provide useful additional information, such data may not always be available for proteins or peptides of unknown structure. In this context, other NMR information in addition to the trans  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar  $^3\text{hJ}$ -couplings would clearly help determine protein and other structures more accurately, particularly as the measurements can be obtained from the same sample. To determine structures from the information, one must be able to perform calculations that accurately relate the structural to the NMR parameters. Clearly, one must be able to experimentally measure the J's with relative

ease and accuracy. Of the three coupling constants that we consider (in addition to the trans  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar  $^3\text{hJ}$ -couplings) the most likely to be useful appears to be the trans H-bond  $^{13}\text{C}$ – $^1\text{H}$  2-bond scalar  $^2\text{hJ}$ -couplings. Although they do vary somewhat with basis set, they are of the same magnitude as the trans  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar  $^3\text{hJ}$ -couplings and they have the virtue of using the major isotope of hydrogen and reasonably abundant isotope of carbon. They are not dominated by the FC terms so much as the other J's that we have considered; however, the calculation of the couplings as the sum of all four contributions has become readily available using Gaussian 03. Although measurement of these couplings poses additional experimental problems, they have been reported for at least one protein.<sup>2</sup> The measured values are reasonably similar to those calculated here. In principle, these couplings could be measured in natural abundance.

Two of the other J's that we have considered both have the experimental advantage of larger coupling magnitudes. However, they both involve  $^{17}\text{O}$ , which is not as experimentally accessible as  $^{13}\text{C}$  (as isotopic labeling would be necessary) and they (like the  $^{13}\text{C}$ – $^1\text{H}$  J's) would require more careful selection of basis set for comparison with theoretical relationships between structure and J than for the  $^{13}\text{C}$ – $^{15}\text{N}$  J's. On the other hand, the FC terms dominate the four contributions to the J's to an even greater extent than for the  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar  $^3\text{hJ}$ -couplings.

The basis set dependence of the first four of the trans H-bond J's that we have considered are much less than one might expect from similar calculations of other J's that have been reported.<sup>18</sup> This observation suggests that moderate sized basis sets will be sufficient for calculations of these trans H-bond J's in reasonably large peptide (and other) structures. The other trans H-bond J's will not be useful for reasons already discussed.

## Conclusions

The trans H-bond  $^{13}\text{C}$ – $^{15}\text{N}$  3-bond scalar J-couplings do not vary significantly with basis set, except for 6-31++G(d,p). They are dominated by the FC term. Thus, unlike many other coupling constants, they can be reasonably estimated with moderate basis sets. As a consequence, calculations on reasonably complex systems can be practically performed.

Other trans H-bond J-couplings could, in principle, lead to additional relationships that might refine the accuracy of structural determinations. Of the three that we considered, the  $^{13}\text{C}$ – $^1\text{H}$  coupling could be measured in natural abundance and is sufficiently invariant with basis set to provide a reliable correlation of calculated and measured J's. The two other trans H-bond J's that we considered both involve  $^{17}\text{O}$ , which would require costly isotopic labeling. These three J's would provide structural information that complements that provide by the  $^{13}\text{C}$ – $^{15}\text{N}$  couplings. Since they can be determined from the same sample under identical conditions, using more than one type of coupling data simultaneously should provide more accurate structural determinations.

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