The *n*-alkane molecules form random coils due to internal rotation about C–C bond, and this tendency increases with increase in chain length, hence the extent of coiling in hexanedecane > decane > hexane. In the ternary *n*-alkane system, hexane, the component of lower surface tension, certainly is enriched in the surface, but even after the surface enrichment possibly the larger decane and hexadecane molecules are so oriented as to cover such a surface fraction that the mixture surface tension is linear vs. bulk mole fraction. The but enrichment by the component of lower surface tension (hexane) is greater in the higher concentration range of hexane, showing larger discrepancies in that region. The principle of corresponding states has been applied successfully to mixtures of *n*-alkanes,[6,18,19] which has been used as a basis in the extension of Flory's statistical theory to enable calculation of surface tension.

Acknowledgment. N.P. thankfully acknowledges the financial assistance from the CSIR, New Delhi, India, for providing a research fellowship. We are grateful to Professor Paul J. Flory for suggestions and help.

Registry No. Hexane, 110-54-3; decane, 124-18-5; hexadecane, 544-76-3.

(18) Prigogine, I.; Bellemans, A.; Naar-Collin, C. *J. Chem. Phys.* **1957** *26*, 751.
(19) Hijmans, J. *Physica* **1961**, *27*, 433.

Interproton Coupling over Five Bonds ^5J(H–C_α–C(O)–N–C_α–H) in the Peptide Moiety: The Importance of Specific Association Effects

M. Barfield,* F. A. Al-Obeidi, V. J. Hruby, and S. R. Walter

Contribution from the Department of Chemistry, University of Arizona, Tucson, Arizona 85721.
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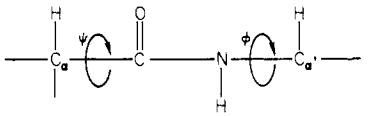
Abstract: An experimental and theoretical study is presented of the conformational and solvent dependencies of long-range H–H coupling constants ^5J(H–C_α–C(O)–N–C_α–H) in compounds which model the peptide backbone. Molecular orbital results for Fermi contact coupling in *cis*- and *trans*-*N*-methylacetamides do not follow a conformational dependence of the homoallylic type; negative values are predicted for most out-of-plane orientations of the C_α–H bonds. In addition, the calculated values for $^5J_{HH'}^{cis}$ and $^5J_{HH'}^{trans}$ are of *opposite* signs in the planar conformation of *cyclo*-(Gly-Gly) and the boat conformation of *cyclo*-(Gly-Tyr). However, relative sign measurements show that these two coupling constants are of the *same* sign in *cyclo*-(Gly-Tyr), and that both are *positive* in *cyclo*-(Gly-Phgly). The inclusion of five water molecules in the MO calculations for *cis*-*N*-methylacetamide and ten water molecules in association with *cyclo*-(Gly-Gly) led to both positive $^5J_{HH'}^{cis}$ and $^5J_{HH'}^{trans}$. As a consequence, any applicability of the empirical relationship of ^5J(H–C_α–C(O–N–C_α–H) to ϕ and ψ angles in peptides does not have any theoretical basis in the molecular oribtal theory for unhydrated amide bonds.

Introduction

In studies of cyclic dipeptides it has been noted[1,2] that the syn coupling constants over five bonds ^5J(H–C_α–C(O)–N–C_α–H) are smaller in magnitude than the anti values.[3] Subsequently, it was proposed[4] that this type of coupling could be diagnostic of syn and anti arrangements about the amide bond. The authors followed this by papers[5] which investigated the dependence of this type of coupling on the dihedral angles ϕ and ψ in compounds which model the peptide backbone **1**. They assumed that the



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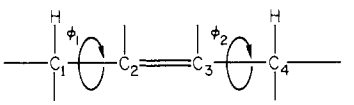
conformational dependencies would be analogous to homoallylic coupling[6] such as would occur in the 2-butene moiety **2**. Hom-



2

oallylic coupling constants have been extensively investigated[6-8] and the π-electronic contributions are satisfactorily represented by the expression

$$^5J^{\pi}_{HH'}(\phi_1,\phi_2) = A \sin^2 \phi_1 \sin^2 \phi_2 + B \qquad (1)$$

where the dihedral angles ϕ_1 and ϕ_2 are measured from the backbone plane as depicted in Figure 1. The calculated MO results[7] for $^5J_{HH'}(\phi_1,\phi_2)$ in *trans*-2-butene, which are plotted in Figure 2 as a function of these two dihedral angles, are also consistent with an angular dependence of the form of eq 1 with $A = 5.0$ Hz and $B \sim 0$. However, eq 1 does not account for the well-documented differences between syn and anti arrangements about the double bond in **2** or the amide bond in **1**.[1,4] These differences can be interpreted in terms of negative contributions from the σ-electron framework in the syn arrangements.[7] It is also interesting to note that replacement of C_2 or C_3 in **2** with

(1) Kopple, K. D.; Ohnishi, M. *J. Am. Chem. Soc.* **1969**, *91*, 962.
(2) For reviews of cyclic dipeptide see: (a) Hruby, V. J. In "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins"; Weinstein, B., Ed.; Marcel Dekker: New York, 1974; Vol. 3, p 1. (b) Anteunis, M. J. O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 627. (c) Bovey, F. A. In "Peptides, Polypeptides, and Proteins"; Blout, E. R., Bovey, F. A., Goodman, M., Lotan, N., Eds.; Wiley: New York, 1974; p 248.
(3) In accord with previous literature for this type of coupling the terms syn and anti will be used to denote the situation in which the C_α and $C_{\alpha'}$ carbon atoms in **1** (C_1 and C_4 carbon atoms in **2**) are on the *same* or *opposite* sides of the amide (double) bond, respectively. In cyclic molecules cis and trans denote protons on the same and opposite sides of the ring, respectively.
(4) Davies, D. B.; Khaled, Md. A. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1651; *Tetrahedron Lett.* **1973**, 2829.
(5) Davies, D. B.; Khaled, Md. A. *J. Chem. Soc., Perkin Trans. 2* **1976**, 187, 1238; 1327.
(6) Karplus, M. *J. Chem. Phys.* **1960**, *33*, 1842, Pinhey, J. T., Sternhell, S. *Tetrahedron Lett.* **1963**, 275.
(7) Barfield, M.; Sternhell, S. *J. Am. Chem. Soc.* **1972**, *94*, 1905.
(8) Jackman, L. M. Sternhell, S., "Applications of NMR Spectroscopy in Organic Chemistry"; Pergamon Press: New York, 1969; Chapter 4-4.

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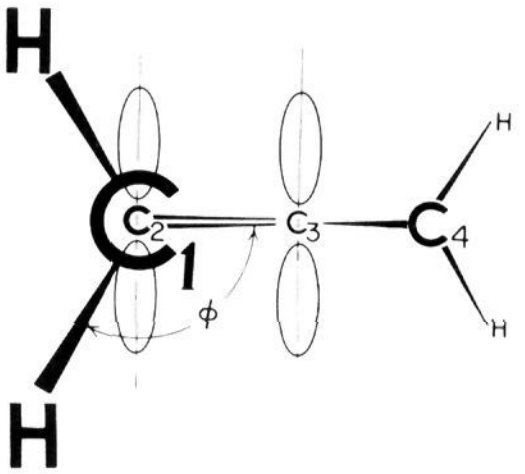


Figure 1. A fragment of the *cis*-2-butene molecule; the dihedral angles ϕ_1 and ϕ_2 are measured in the same sense from the C_1–C_2–C_3 plane.

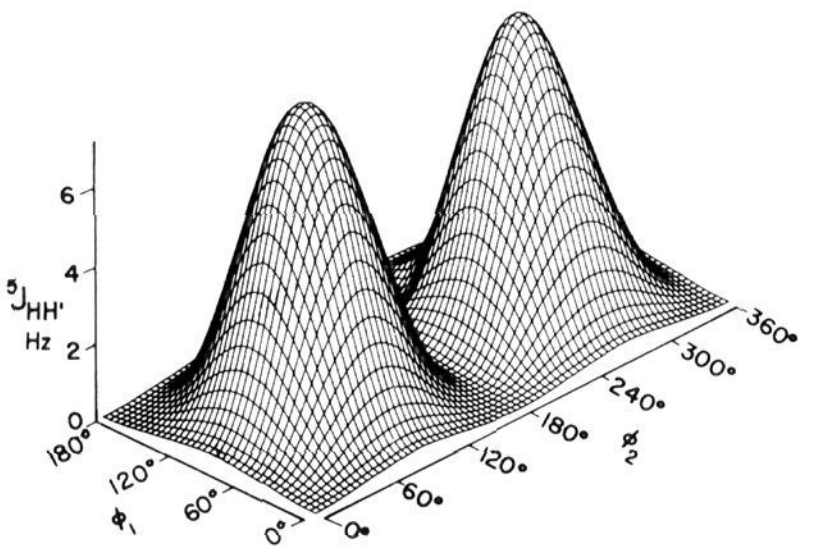


Figure 2. Calculated INDO–FPT MO results for homoallylic H–H coupling in *cis*-2-butene plotted as a function of the dihedral angles ϕ_1 and ϕ_2. The dihedral angles are defined in Figure 1. The data are from ref 7.

sp^2 hybridized nitrogen does not produce any major changes in homoallylic coupling.[7]

In NMR studies of amides and cyclic dipeptides it was assumed[5] that ^5J(H–C_α–C(O)–N–C_αH) would have an angular dependence of the form of homoallylic coupling according to eq 1 with $A = nA^s$, and $B = 0$, where n is the number of equivalent coupling paths ($n = 2$ for cyclic dipeptides) and A^s is a constant which is dependent on solvent ($A^s = 1.37$ Hz in D_2O and 0.87 Hz in Me_2SO). The decrease in A below the homoallylic value was attributed to the partial double-bond character of the amide bond, but it was suggested that the molecular orbital (MO) results (vide infra) would account for the fine details of long-range coupling across the amide bond. No explanation for the solvent dependence of A^s was given.[5]

It has been noted that the possible conformations of cyclic dipeptides in solution (flagpole–boat, planar, and bowsprit boat) may also be elucidated by the vicinal H–N–C_α–H and geminal H–C_α–H coupling constants in the glycyl residues.[1-5,9] However, is has been suggested[2c] that the vicinal H–N–C_α–H couplings are sometimes inconsistent with the X-ray diffraction structures of cyclic dipeptides.

It is the purpose of this study to determine the theoretical basis for the empirical relationship between ^5J(H–C_α–C(O)–N–C_α–H) and the dihedral angles ϕ and ψ in the peptide moiety. Some serious disparities between the MO results and the form of eq 1 prompted experimental studies of the signs of these long-range coupling constants and the importance of specific association effects.

Calculated $^5J_{HH'}$ in Isolated *N*-Methylacetamides and Cyclic Dipeptides. Molecular orbital (MO) results for the Fermi contact contributions to the long-range coupling constants ^5J(H–C_α–C(O)–N–C_α–H) were based on the FPT (finite perturbation theory) method in the INDO (intermediate neglect of differential overlap) approximation of self-consistent-field MO theory.[10,11] Molecular geometries were based on the Corey–Pauling model.[12] Calculated results for ^5J(H–C_α–C(O)–N–C_α–H) in *trans*-*N*-methylacetamide(**3**) are plotted in Figure 3 as a function of the dihedral angles θ_1 and θ_2.[13] These dihedral angles are measured from the backbone atoms as depicted in Figure 4. The calculated values range from +0.25 to –0.55 Hz and are negative for most nonplanar arrangements. By way of contrast, the homoallylic H–H coupling constants in *trans*-2-butene in Figure 2 are all positive. A comparison of the data is Figures 2 and 3 shows that not only are the long-range coupling constants over the amide bond reduced in magnitudes (as might be expected from the decrease in π-bond order), but the signs of the coupling constants are *opposite* for most values of the out-of-plane conformations!

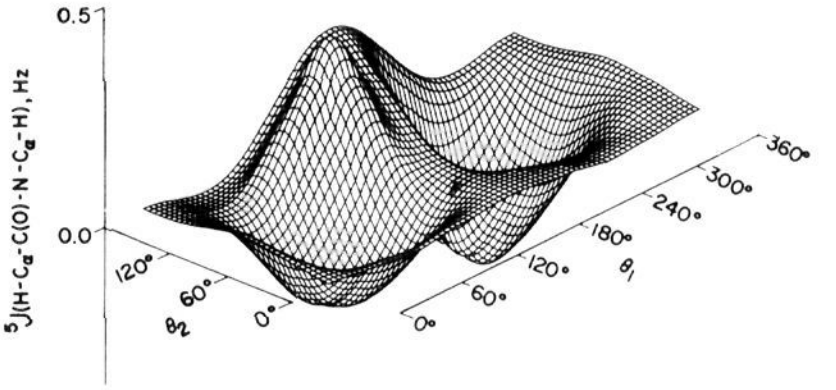


Figure 3. Calculated INDO–FPT MO results for ^5J(H–C_α–C(O)–N–C_α–H) in *trans*-*N*-methylacetamide plotted as a function of the dihedral angles θ_1 and θ_2, which are defined in Figure 4.

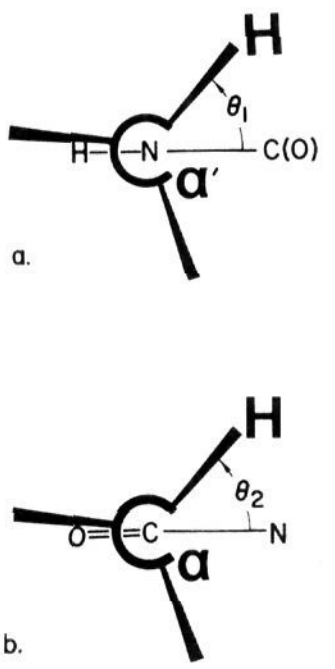
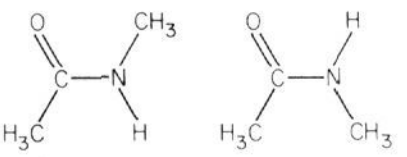


Figure 4. Designations of the dihedral angles θ_1 and θ_2 in the peptide moiety. Since the substituents at C_α and $C_{\alpha'}$ were the same in all of the MO calculations, chiral designations were not adopted.

Molecular orbital calculations were also performed for ^5J(H–C_α–C(O)–N–C_α–H) in *cis*-*N*-methylacetamide(**4**) as a function



3 **4**

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(11) Dobosh, P. A. Quantum Chemistry Program Exchange, Program No. 142, modified for coupling constant calculations by M. Barfield. To perform the calculation for *cyclo*-(Gly-Gly)·10H_2O it was necessary to modify the program to handle 44 atoms and 98 orbitals.

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(13) Since the calculations were performed at 30° intervals of the dihedral angles, a four-point interpolation program was written to prepare the plots at 5° intervals of the dihedral angles.

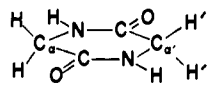
Table I. Calculated INDO-FPT MO Results for the Long-Range ^5J(H-C_α-C(O)-N-C_α-H) and Vicinal ^3J(H-N-C_α-H) Coupling Constants in *cyclo*-(Gly-Gly) and *cyclo*-(Gly-Tyr)[a]

coupling constant	*cyclo*-(Gly-Gly)	*cyclo*-(Gly-Tyr)	
	5a	5b[b]	6
$^5J_{HH'}^{cis}$	0.12	−0.03 { 0.42, −0.48 }	0.54 (0.88)[c]
$^5J_{HH'}^{trans}$	−1.21	−0.82 { −0.84, −0.81 }	−0.74 (1.36)[c]
^3J(H-N-$C_{\alpha'}$-H_s')	1.40 (2.2)[d]	0.88[e] { 1.70, 0.06 }	1.62
^3J(H-N-C_α-H_s)	1.40 (2.2)[d]	1.24 { 2.47, 0.01 }	2.39 (3.0)[d]
^3J(H-N-C_α-H_b)	1.40 (2.2)[d]	1.24 { 2.47, 0.01 }	−0.02 (≤0.5)[d]

[a] All values in hertz. [b] Averaged values for the interconverting boat forms of *cyclo*-(Gly-Gly). [c] This work; the signs of the experimental values are assumed to be positive as discussed in the text. [d] Values measured in Me_2SO-d_6. [e] This value differs from the other two values of ^3J because the calculation was based on X-ray data for 6, which does not have the planar symmetry of 5a.

of θ_1 and θ_2. The calculated syn coupling constants cover a larger range of values than the anti values (Figure 3), and there are so many variations that a three-dimensional plot is difficult to interpret and is not reproduced here. Some representative calculated values of long-range coupling constants in the syn arrangement are as follows: $^5J(0°,0°) = -1.61$, $^5J(60°,60°) = -0.73$, $^5J(60°,300°) = -0.23$, $^5J(180°,180°) = 0.37$, $^5J(120°,120°) = -0.37$, $^5J(120°,240°) = -0.41$ Hz. It is important to note that coupling constants of negative sign, which occur for the out-of-plane orientation of the hydrogen atoms in **5** and **6**, are incompatible with the theoretical models[6,7] which led to eq 1. If alternative mechanisms for transmission of the coupling over the amide bond are important, then the applicability of eq 1 is fortuitous.

Since the empirical relationship[5] or ^5J(H-C_α-C(O)-N-C_α-H) to dihedral angles was based on the data for cyclic dipeptides, it was also of interest to obtain INDO-FPT results for the planar **5a** and boat **5b** conformations of *cyclo*-(Gly-Gly) and *cyclo*-



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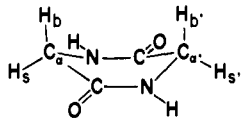
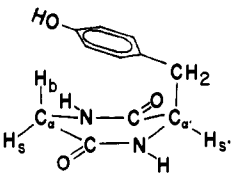


5b

(Gly-Tyr) (**6**). The calculated values for the long-range and



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vicinal H-H coupling constants are entered in Table I. Available experimental data are given in parentheses. the geometries for *cyclo*-(Gly-Gly) (**5a**) and *cyclo*-(Gly-Tyr) (**6**) were based on X-ray diffraction data.[14,15] In solution there is a possibility that *cyclo*-(Gly-Gly) interconverts between two equivalent boat forms. To investigate the implications of this on the signs of the coupling constants, INDO-FPT MO calculations were also performed for **5b**. Geometrical data were based on the X-ray results for *cyclo*-(Gly-Tyr).[15] The long-range and vicinal coupling constant data, which were obtained as averages, are entered in Table I. Because of the asymmetry of the diketopiperazine ring in **6**, one of the calculated vicinal coupling constants is different. More importantly, the averaging of the boat conformations produces no major changes in the five-bond H-H coupling constants; the cis value is small and negative. Note that the inclusion of the tyrosine side chain increases the calculated long-range coupling constants by only 0.1 Hz. Also, it is of some interest to note that the calculated vicinal coupling constant data in Table I are in reasonable agreement with the experimental values measured in Me_2SO.[1]

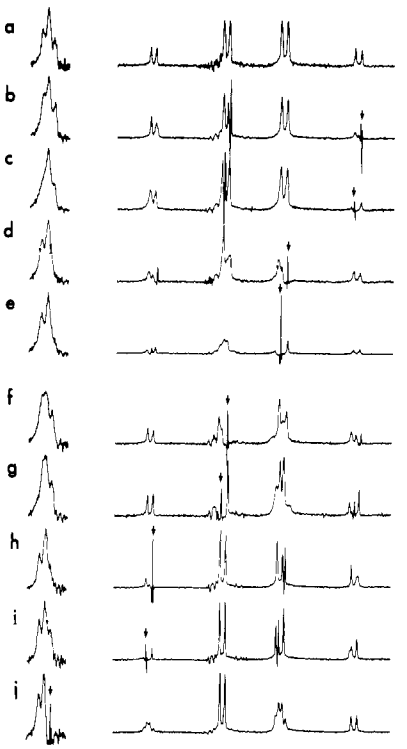


Figure 5. Spin-tickling results for *cyclo*-(Gly-Phgly) in D_2O. (a) The normal ABX spectrum at 250 MHz (the phenyl protons resonances are not shown). The X part of the spectrum is at higher frequency. The splittings in the AB part of the spectrum are 1.47, 1.66, and 18.51 Hz. (b-j) Spectra obtained on irradiation of the lines indicated by the arrows.

The most important features of the calculated data in Table I are that the $^5J_{HH'}^{cis}$ are quite sensitive to the conformation of the diketopiperazine ring, exhibiting both positive and negative values, and the $^5J_{HH'}^{trans}$ are negative and exhibit smaller relative changes. The possibility of cis and trans long-range H-H coupling constants of opposite signs over dual paths has been noted elsewhere.[16,17] More recently, homoallylic coupling constants of *negative* sign have been proposed[18] in situations in which the π-bond order is decreased substantially below unity. As a consequence, relative sign determinations in appropriate amides and cyclic dipeptides are of interest.

Relative Signs of ^5J(H-C_α-C(O)-N-C_α-H) in *cyclo*-(Gly-Tyr) and *cyclo*-(Gly-Phgly). Proton magnetic resonance spectra of *cyclo*-(Gly-Tyr) were measured in D_2O with the pD adjusted to approximately 3 as sharper lines are obtained in the slow-exchange region. Assuming that the low-frequency resonance of the glycyl residue arises from the proton cis to the tyrosine side chain,[1] the cis and trans $^5J_{HH'}$ have magnitudes 0.88 and 1.36 Hz, respectively, and $^2J_{HH'} = -18.39$ Hz. The use of "spin-tickling" nuclear magnetic double resonance (NMDR) techniques[19,20] demonstrated

(14) Deigeilh, R.; Marsh, R. E. *Acta Crystallogr.* **1959**, *12*, 1007.
(15) Lin, C.-F.; Webb, L. E. *J. Am. Chem. Soc.* **1973**, *95*, 6803.
(16) Barfield, M.; Spear, R. J.; Sternhell, S. *J. Am. Chem. Soc.* **1975**, *97*, 5160.
(17) Barfield, M.; Spear, R. J.: Sternhell, S. *J. Am. Chem. Soc.* **1971**, *93*, 5322.
(18) Barfield, M.; Abia, A. A. *Org. Magn. Reson.* **1980**, *14*, 404.
(19) Freeman, R.; Anderson, W. A. *J. Chem. Phys.* **1962**, *37*, 2053.

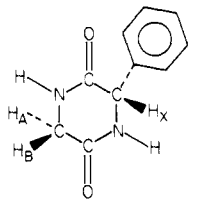
Table II. Change in the Values of $^5J^{cis}_{HH'}$, $^5J^{trans}_{HH'}$, and $^2J_{HH'}$ in *cyclo*-(Gly-Phgly) (7) for Various Concentrations of D_2O and Acetone-d_6[a]

vol fraction of acetone-d_6 in D_2O	$^5J^{cis}_{HH'}$	$^5J^{trans}_{HH'}$	$^2J_{HH'}$
0.00	1.66	1.47	−18.51
0.31	1.32	1.25	−18.48
0.50	1.05	1.10	−18.38
0.71	0.90	0.98	−18.26
0.97	0.73	0.83	−18.07
1.00	0.56	0.73	−17.58

[a] All coupling constants in hertz.

that the long-range coupling constants are of the *same* sign, but the complexity of the spin system in combination with a number of small coupling constants prevented an unambiguous determination of these signs relative to the (negative) geminal H–H coupling constant.[21]

To obtain a simpler NMR system, *cyclo*-(Gly-Phgly) (**7**) was



7

prepared (see Experimental Section) and the ABX-type NMR spectra were also examined in D_2O with pD ~3. The magnitude of the cis and trans long-range coupling constants are 1.66, and 1.47 Hz, respectively, assuming that the resonance at lowest frequency arises from the proton which is trans to the phenyl. The ABX part of the NMR spectrum of **7** is shown in Figure 5a. "Spin-tickling" experiments were performed by irradiating every line; representative spectra with spin tickling of transitions marked by an arrow are given in Figure 5b–j. These results show that the two long-range coupling constants are of the same sign, but they are opposite in sign to the geminal H–H coupling constant (−18.51 Hz). Therefore, the cis and trans long-range coupling constants are both positive, in disagreement with the calculated MO results in Table I for the trans coupling constants of *cyclo*-(Gly-Gly) and *cyclo*-(Gly-Tyr). Unfortunately, the structure of *cyclo*-(Gly-Phgly) is not yet known. However, the geminal and vicinal H–N–C_α–H coupling constants, measured in Me_2SO,[1] are almost identical with those for *cyclo*-(Gly-Tyr) and imply a boat or twist boat conformation.[1] The large difference in the magnitudes of the long-range cis coupling constants suggests that there is some conformational change in the phenylglycine residue. However, in view of the many other similarities in coupling constants, it is quite possible that $^5J^{cis}_{HH'}$ and $^5J^{trans}_{HH'}$ in *cyclo*-(Gly-Tyr) are also positive.

Solvent Dependence of ^5J(H–C_α–C(O)–N–C_α–H). To investigate the possibility of a solvent dependent change in the signs of the long-range coupling constants, the proton magnetic resonance spectra of *cyclo*-(Gly-Phgly) were measured in a series of mixed solvents having varying concentrations of D_2O in acetone-d_6. The $^5J^{cis}_{HH'}$ and $^5J^{trans}_{HH'}$ and $^2J_{HH'}$ are entered in Table II for volume fractions of the two solvents ranging from neat D_2O to neat acetone-d_6. The magnitude of the long-range coupling constants decreases substantially in changing from neat D_2O to neat acetone-d_6 as solvent; $^5J^{cis}_{HH'}$ and $^5J^{trans}_{HH'}$ decrease by factors of about 3 and 2, respectively. Clearly, this type of long-range H–H coupling constant is dominated by the nature of the solvent, but the monotonic decreases in the magnitudes are not indicative of a change in sign. The geminal H–H coupling constants, which

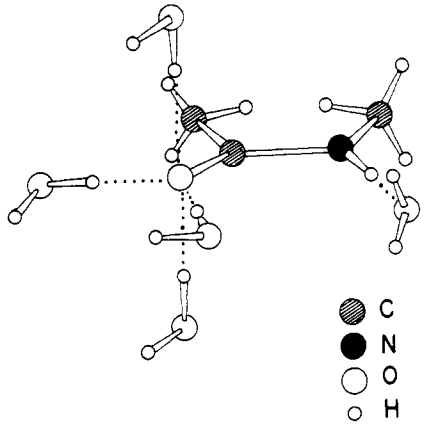


Figure 6. Geometry of a fully hydrated model for *cis-N*-methylacetamide. The five water molecules were arranged according to the model proposed for *trans-N*-methylacetamide.[26]

are also quite sensitive to the polarity of the medium,[22] and to conformational factors, increase by less than 1 Hz.

Calculated Dependence of ^5J(H–C_α–C(O)–N–C_α–H) in *cis-N*-Methylacetamide on Hydrogen-Bonding Effects. Theoretical calculations are almost invariably carried out for isolated molecules, whereas NMR data are almost always obtained in condensed phases. Theoretical studies of solvent dependencies of nuclear spin–spin coupling constants[22,23] have emphasized the role of aprotic solvents in relatively rigid solutes. A few calculations of directly bonded ^{13}C–^1H coupling constants have been performed[24] in cases in which specific association effects, i.e., hydrogen-bonding effects, may be important.

Because of the importance of solute–solvent interactions for protein structure and stability, a large number of MO calculations have been performed to investigate the structure of amide–water complexes.[25,26] To investigate the importance of amide–water interactions on ^5J(H–C_α–C(O)–N–C_α–H), a model was assumed for *cis-N*-methylacetamide (**4**) surrounded by five waters of hydration. The geometrical arrangement, which is depicted in Figure 6, was based on the one optimized for *trans-N*-methylacetamide.[26] Although the Corey–Pauling geometry for **4** was retained, the distances and orientations of the water molecules relative to the amide function were based on those given in ref 26. With these assumptions the calculated INDO–FPT results for the cis and trans H-H coupling constants in the amide–$5H_2O$ complex are 0.00 and 0.01 Hz in comparison with the values −0.37 and −0.41 Hz, respectively, for the isolated amide. Changes of this order or magnitude were unexpected, so that additional calculations were suggested; the O···H hydrogen bonding distances were optimized in INDO calculations performed for a formamide–water trimer (1.43 and 1.53 Å were obtained in comparison with the values of 1.68 and 1.74 Å in the model of Scheiner and Kern[26]); for *cis-N*-methylacetamide with five waters of hydration the calculated cis and trans ^5J(H–C_α–C(O)–N–C_α–N) were both +0.12 Hz. The electronic factors which produce a change in the signs of the calculated coupling constants are not yet understood. At least qualitatively, the more positive values of the coupling constants with increasing hydration of the amide bond are consistent with experimental trends in Table II and the positive values for cis and trans coupling constants in **7**. A more appropriate theoretical model would incorporate the statistically averaged configurational properties of solvent and solute.[27]

Since the calculated values of $^5J^{cis}_{HH'}$ and $^5J^{trans}_{HH'}$ in Table I are opposite in sign (or both negative), it is of interest to investigate

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the importance of specific association effects in cyclic dipeptides. For this purpose an INDO–FPT MO calculation was performed for the planar arrangement of *cyclo*-(Gly-Gly)·10H_2O. The ten water molecules were oriented about the two amide functional groups in the manner depicted in Figure 6 for *N*-methylacetamide. Both of the long-range coupling constants were found to be positive; i.e., $^5J^{cis}_{HH'}$ = 1.82 Hz and $^5J^{trans}_{HH'}$ = 0.06 Hz. Therefore, specific association effects, which are associated with the inclusion of the ten water molecules in the MO calculation, increase the cis and trans coupling constants by 1.7 and 1.3 Hz, respectively. In looking for the electronic factors leading to these relatively large changes, it should be noted that the amide π-bond orders increase from 0.49 in **5a** to 0.59 in *cyclo*-(Gly-Gly)·10H_2O. Similarly, the increased magnitude of ^5J(H–C_α–C(O)–N–C_α–H), measured in trifluoroacetic acid, have been attributed to an increase in bond order associated with protonation of the amide bond.[28]

Conclusions

The factors which determine the conformational dependencies of the long-range ^5J(H–C_α–C(O)–N–C_α–H) coupling constants are more complicated than suggested by the simple analogy to homoallylic H–H coupling constants.[5] The calculated signs and magnitudes of these long-range coupling constants are dependent on changes in the electronic character of the amide group due to specific association effects with solvent molecules. The MO results indicate that the long-range coupling constants tend to become more positive if specific association effects, arising from waters of hydration, are included in the computations. This is consistent with the observation of positive values for $^5J^{cis}_{HH'}$ and $^5J^{trans}_{HH'}$ for *cyclo*-(Gly-Phgly) and the monotonic decrease in these coupling constants in less polar solvents. These findings may have implications for other types of coupling involving the amide bond,[29] and provide an extreme example of a physical situation in which calculations for isolated molecules are completely inapplicable to the molecules in solution. The applicability of the empirical relationship,[5] which is based on analogy to homoallylic coupling constants, appears fortuitous.

Experimental Section

A. Spectra. NMR results for *cyclo*-(Gly-Tyr) and *cyclo*-(Gly-Phgly) were obtained at 250 MHz on a Bruker Instruments WM-250 FT NMR spectrometer. Spectra were recorded in D_2O with pD adjusted to about 3 or in acetone-d_6, which served as internal lock materials. In all cases the digital resolution was at least 0.05 Hz per point. Resolution enhancement was performed by Gaussian multiplication. The coupling constants for *cyclo*-(Gly-Phgly) in Table II were measured directly. The NMR parameters for *cyclo*-(Gly-Tyr) were based on an iterative spectral analysis using Bruker Instruments' PANIC 80 NMR simulation program.[30] The root-mean-square error was 0.022 Hz. Infrared spectra were recorded on a Perkin-Elmer 398 spectrometer; optical activity measurements were performed with a Perkin-Elmer 241MC polarimeter, and the routine NMR spectrum of **7** was recorded on a Varian Associates T-60 NMR spectrometer.

B. Syntheses. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected.

D-Phenylglycine Methyl Ester Hydrochloride. D-Phenylglycine (1 g, 1.9, mmol) was mixed with (10 mL) of anhydrous methyl alcohol at −5 °C, and 8 mL of thionyl chloride (13.3 g, 198 mmol) was added dropwise so that the temperature remained below 0 °C.[31] The methyl ester hydrochloride was recrystallized from methanol/ether: yield 1.2 g (92%), mp 208 °C (lit.[32] mp 202 °C).

Methyl N^α-Boc-glycyl-D-phenylglycinate. Boc-Gly (0.347 g, 1.98 mmol) was dissolved in 20 mL of anhydrous tetrahydrofuran, and 0.276 mL of anhydrous triethylamine (1.98 mmol) was added. The mixture was cooled to −20 °C under nitrogen. To the stirring mixture of Boc-Gly·Et_3N, pivaloyl chloride (0.244 mL, 1.98 mmol) was added dropwise with stirring. The mixed anhydride was stirred at −20 °C for 10 min and then a solution of phenylglycine methyl ester hydrochloride (0.4 g, 1.98 mmol) containing anhydrous Et_3N (0.276 mL, 1.98 mmol) in anhydrous dimethylformamide was added slowly. The resulting mixture was stirred for 1.5 h until it reached room temperature. The completion of the reaction was checked by a ninhydrin test. the triethylamine hydrochloride was filtered off and the filtrate washed with three 20-mL portions of water, 5% $NaHCO_3$, 1 N citric acid, and water, dried over anhydrous $MgSO_4$, and evaporated under vacuum to dryness. The oily residue of the dipeptide failed to recrystallize from several different solvent systems, yield 0.6 g (94%).

***cyclo*-Glycyl-D-phenylglycyl.** The oily residue of Boc-Gly-D-Phgly-OMe (0.6 g, 1.85 mmol) was dissolved in formic acid (20 mL, 98%) and the solution was stirred at room temperature for 3 h.[33] The formic acid was removed in vacuo at 30 °C on a rotary evaporator, and the resulting formate salt of the dipeptide ester was dissolved in *sec*-butyl alcohol (30 mL) and toluene (8 mL). The mixture was boiled for 3 h, and a further 10 mL of *sec*-butyl alcohol was added. The solution was concentrated and cooled to 0 °C and the precipitated product was recrystallized from aqueous methanol (ethyl acetate–petroleum ether can also be used) to give 0.28 g, 80% based on dipeptide: mp 240–241 °C (lit.[1] mp 241–243 °C) dec; ^1H NMR δ (Me_2SO-d_6) 2.2 (s, variable), 3.8 (br d, 2 H), 4.81 (br s, 1 H), 7.28 (s, 5 H); IR (KBr) 3520, 1650 cm^{-1}; $[\alpha]^{23}_D$ −696° (*c* 0.05, Me_2SO). Anal. Calcd for $C_{10}H_{10}N_2O_2 \cdot {}^1/_4H_2O$: C, 61.6; H, 5.2; N, 14.4. Found: C, 61.7; H, 5.3; N 14.5.

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