Influence of the Nonplanarity of the Amide Moiety on Computed Chemical Shifts in Peptide Analogs. Is the Amide Nitrogen Pyramidal?

Horst M. Sulzbach,[1a,b] Paul v. R. Schleyer,*[,1a,b] and Henry F. Schaefer, III*[,1a]

Contribution from the Center for Computational Quantum Chemistry, the University of Georgia, Athens, Georgia 30602, and Computer Chemistry Center, Institute of Organic Chemistry I, Universität Erlangen-Nürnberg, Henkestr. 42, D-91054 Erlangen, Germany

Received June 6, 1994[⊗]

Abstract: The dependence of peptide NMR chemical shifts on the nonplanarity of the amide nitrogen environment has been studied *ab initio*. At both the GIAO-SCF and the correlated GIAO-MP2 levels, each 10° deviation from 180° of the $H^t-N-C^\circ-O$ dihedral angle of formamide deshields the carbonyl carbon (C°) by about 2 ppm. The NMR chemical shift of the amide nitrogen, however, remains almost constant when the $H^t-N-C^\circ-O$ dihedral angle is between 140° and 180°. An α-helical (**1a**) and a β-pleated sheetlike structure (**1b**) of *N*-formylglycine amide (**1**) were optimized at different levels of theory. The RMP2-FC/6-31G*-, RHF/6-31G*-, and AM1-optimized geometries of the α-helical structure have an $H^t-N-C^\circ-O$ dihedral angle of about −160°, and the calculated Δδ C° deviates by only 1−3 ppm from the experimental range. However, Δδ C° for the RHF /3-21G geometries, which have a planar amide moiety in both an α-helical and a β-pleated sheetlike structure, deviates by about 8 ppm from the experimental range. Δδ C° between an α-helical and a β-pleated sheetlike structure was computed for *N*-acetyl-*N'*-methylglycine amide (**2**) at the GIAO-SCF/6-311G*//AM1 level as a function of the $H^t-N-C^\circ-O$ dihedral angle. The experimental range (2−5 ppm) for $\Delta\delta\ ^{13}C\ C^\circ$ is reached when the dihedral angle is smaller than −160°, whereas $\Delta\delta\ ^{13}C\ C^\alpha$ and $\Delta\delta\ ^{15}N\ N^{amide}$ do not depend significantly on $H^t-N-C^\circ-O$. The differences in chemical shifts of C° for the smaller *N*-formyl-*N'*-methylglycine amide (**3**) computed with both the GIAO-SCF and GIAO-MP2 methods for α-helical and β-pleated sheetlike conformations increase by 1.5 ppm for each 10° deviation from 170° of the $H^t-N-C^\circ-O$ dihedral angle. Semiempirical computations on a model α-helix consisting of eight glycine residues give a $H^t-N-C^\circ-O$ dihedral angle of −164° in good agreement with the *ab initio* results for our dipeptide models.

Introduction

NMR is important in helping deduce conformations of polypeptides and proteins in solution. Generally, only nuclear Overhauser effect (NOE) information and coupling constants are employed.[2] Recently, there has been increasing interest in the possible additional application of NMR chemical shift information for the same purpose.[3-7] Knowledge of the relationship between chemical shifts and polypeptide conformations is needed, but this can now be obtained computationally, *e.g.*, by the IGLO[8-12] or GIAO[13-17] methods. The changes in the chemical shifts for C^α and C^β among various conformations can be determined by computing the chemical shielding "surface" with respect to ϕ and ψ (the dihedral angles that define the backbone conformation of peptides,[18] see Figure 1). The computations are facilitated further because relative changes in the chemical shifts of glycine and glycine amide,[19] as a function of the backbone angle ψ, are almost the same at the correlated GIAO-MP2 as at the GIAO-SCF level of theory.

The evaluation of a large amount of NMR data on proteins with known structures has demonstrated that $\delta\ ^{13}C$ for C^α and C° in an α-helical region is shifted downfield by 2−5 ppm compared to the signals in a β-pleated sheet.[20-22] C^β is shifted upfield by roughly the same amount. *Ab initio* studies on peptide models[3-7] were able to reproduce the experimental differences in the chemical shifts for C^α and C^β between α-helical and β-pleated sheetlike structures within 2−3 ppm.

Calculations of the differences in the NMR chemical shifts between two conformations of the same molecule are quite

* Correspondence should be addressed to the Center for Computational Quantum Chemistry, Athens, GA 30602.

⊗ Abstract published in *Advance ACS Abstracts*, February 15, 1995.

(1) (a) University of Georgia. (b) Universität Erlangen-Nürnberg.
(2) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
(3) Jiao, D.; Barfield, M.; Hruby, V. J. *J. Am. Chem. Soc.* **1993**, *115*, 10883.
(4) De Dios, A. C.; Pearson, J. G.; Oldfield, E. *Science* **1993**, *260*, 1491.
(5) De Dios, A. C.; Pearson, J. G.; Oldfield, E. *J. Am. Chem. Soc.* **1993**, *115*, 9768.
(6) Laws, D. D.; de Dios, A. C.; Oldfield, E. *J. Biomol. NMR* **1993**, *3*, 607.
(7) Jiao, D.; Barfield, M.; Hruby, V. J. *Magn. Reson. Chem.* **1993**, *31*, 75.
(8) Kutzelnigg, W. *Isr. J. Chem.* **1980**, *19*, 193.
(9) Schindler, M.; Kutzelnigg, W. *J. Chem. Phys.* **1982**, *76*, 1919.
(10) Schindler, M.; Kutzelnigg, W. *J. Am. Chem. Soc.* **1983**, *105*, 1360.
(11) Fleischer, U.; Schindler, M. *Chem. Phys.* **1988**, *120*, 103.
(12) Kutzelnigg, W.; Fleischer, U.; Schindler, M. *NMR Basic Principles and Progress*; Springer-Verlag: Berlin, 1990; Vol. 23.
(13) Ditchfield, R. *MTP International Review of Science Physical Chemistry Series I. Molecular Structure and Properties*; Butterworth: London, 1972.
(14) Ditchfield, R. *Mol. Phys.* **1974**, *27*, 789.
(15) Ditchfield, R.; Ellis, P. D. In *Topics in Carbon-13 NMR Spectroscopy*; Levy, G. C., Ed.; Wiley: New York, 1974.
(16) Ditchfield, R. *J. Chem. Phys.* **1972**, *56*, 5688.
(17) Pulay, P.; Wolinski, K.; Hinton, J. F. *J. Am. Chem. Soc.* **1990**, *112*, 8251.
(18) Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. *J. Mol. Biol.* **1963**, *7*, 95.
(19) Sulzbach, H. M.; Schleyer, P. v. R.; Schaefer, H. F. *J. Am. Chem. Soc.* **1994**, *116*, 3967.
(20) Kricheldorf, H. R.; Müller, D. *Macromolecules* **1983**, *16*, 615.
(21) Spera, S.; Bax, A. *J. Am. Chem. Soc.* **1991**, *113*, 5490.
(22) Reily, M. D.; Thanabal, V.; Omecinsky, D. O. *J. Am. Chem. Soc.* **1992**, *114*, 6251.

0002-7863/95/1517-2632$09.00/0 © 1995 American Chemical Society

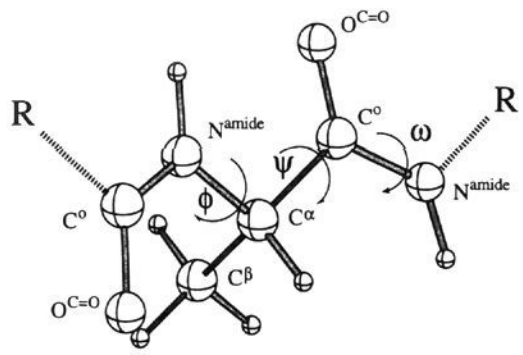


Figure 1. Definition of the backbone angles ϕ and ψ, shown for an alanine residue ($\phi = \psi = 180°$). The dihedral between C°−N−C$^{\alpha}$−C° is designated ϕ, and the dihedral between N−C$^{\alpha}$−C°−N is designated ψ. The eclipsed conformer has $\phi = 0°$ and $\psi = 0°$ Rotation to the right is counted positive.

accurate. Any systematic error affects both structures and tends to cancel when chemical shift differences between the two conformations are taken. Hence, it is surprising that the $\Delta\delta$ ^{13}C value between an α-helical and a β-pleated sheetlike structure of GD of −4.5 ppm, which Jiao *et al.* computed for C°; deviates significantly from the experimental range ($\Delta\delta$ ^{13}C = 2−5 ppm).[3] What is the reason?

Sulzbach *et al.* have shown that the effect of electron correlation on the computed NMR chemical shifts is almost the same for different conformations of the same molecule.[19] Hence, correlation effects cannot be responsible for the poor description of $\Delta\delta$ ^{13}C of C°, which was obtained for GD by Jiao *et al.* Chestnut *et al.* have theoretically found that the effect of hydrogen bonding at the carbonyl oxygen changes the NMR chemical shielding of the carbonyl carbon in glycylglycine by only 2.3−4.0 ppm.[23] If either the α-helix or the β-pleated sheet forms more hydrogen bonds, the effect of hydrogen bonding on the NMR chemical shifts will influence the difference in $\Delta\delta$ between both conformations. However, hydrogen bonding involving the carbonyl oxygen atom is present in both the α-helix and the β-pleated sheet, and the contribution to $\Delta\delta$ that is due to different degrees of hydrogen bonding in both structures should only be a fraction of 2.3−4.0 ppm. This does not seem large enough to explain the observed discrepancy between experiment and theory. Most likely, Jiao *et al.* used inaccurate geometries for GD for their chemical shift computations.[3] The bond lengths and bond angles of GD are well-known and change little between different levels of theory. For the computation of the NMR chemical shielding, the backbone dihedral angles, ϕ and ψ of GD, are constrained to the values found in an α-helix and a β-pleated sheet. Hence, the dihedral angles of the amide moiety are the only geometrical parameters that might be described poorly. Nonplanarity of the amide group could influence the NMR chemical shifts, and this might be responsible for the $\Delta\delta$ C° deviations.

The amide nitrogen is expected to have a planar environment due to the partial carbon−nitrogen double bond. However, various experimental and theoretical studies have come to contradicting conclusions regarding the planarity of formamide. Nevertheless, they agree that the force constant for out-of-plane bending at N is very small.[24−30] Although urea is planar in the crystal,[31−34] Rasul *et al.*, in agreement with earlier RHF results,[35] computed the minimum to have C_2 symmetry with a H^{t}−N−C°−O dihedral angle of about −150° at various correlated levels.[36] Correlated *ab initio* computations predict the amino nitrogen in the DNA bases to be nonplanar.[37]

All prior computations of peptide NMR chemical shifts assumed the amide nitrogen to have a planar environment. The geometries employed in the chemical shift computations were obtained from empirical force field programs,[5] low-resolution X-ray structures,[4,6] or *ab initio* optimizations at the RHF/3-21G level,[3] which generally does not describe the amide group correctly.[29,30]

De Dios *et al.* found that the various parameters that affect the chemical shifts (bond lengths, bond angles, and dihedral angles) of glycine, alanine, and valine residues are not strongly coupled and therefore can be treated additively.[5] If the nonplanarity of the amide nitrogen influences the chemical shifts, the degree of out-of-plane bending of the nitrogen (dependence of $\Delta\delta$ on the H−N−C°−O dihedral angle is a convenient measure) will have to be considered as an additional parameter.

Formamide is the simplest model amide. However, in peptides and proteins only a single hydrogen is bound to the amide nitrogen, and the amide moiety is subject to many additional structure-determining effects, *e.g.*, the secondary structure of the neighboring residues. Hence, a dipeptide is a more pertinent model for studying the amide nitrogen geometry. However, the size of dipeptides limits the level of theory that can be employed. We determined the influence on the NMR chemical shielding of a non-planar nitrogen environment for formamide at very high levels of theory. *N*-Formylglycine amide (**1**), *N*-acetyl-*N*′-methylglycine amide (**2**), and *N*-formyl-*N*′-methylglycine amide (**3**) were employed as peptide model systems. Both **1** and **2** have often been used for this purpose.[38] *N*-Formyl-*N*′-methylglycine amide, which has the methyl group at the amide nitrogen like **2** and is considerably less demanding to study theoretically, allowed us to evaluate the chemical shifts at the GIAO-MP2/6-311G level.

The possible non-planar nitrogen environment can also be verified by employing semiempirical methods to study small peptide models. Such models have already been used to elucidate the reaction mechanisms at the reactive centers of enzymes.[39]

We address the following questions in this study: (1) How sensitive are the NMR chemical shifts, especially of C°, to a change in the degree of nonplanarity of the nitrogen environment? (2) How large is the H−N−C°−O dihedral angle, which is a measure of the nonplanarity of the amide function, in **1** and **2**? (3) Is a peptide model, which assumes a pyramidal nitrogen environment, in agreement with experimental data for peptides?

(23) Chestnut, D. B.; Phung, C. G. A. In *Nuclear Magnetic Shieldings and Molecular Structure*; Tossell, J. A., Ed.; Kluwer Academic Publishers: Boston, 1993.
(24) Kurland, R. J. *J. Chem. Phys.* **1955**, *23*, 2202L.
(25) Kurland, R. J.; Wilson, E. B. *J. Chem. Phys.* **1957**, *27*, 585.
(26) Costain, C. C.; Dowling, J. M. *J. Chem. Phys.* **1960**, *32*, 158.
(27) Hirota, E.; Sugisaki, R.; Nielsen, C. J.; Sørensen, G. O. *J. Mol. Spectrosc.* **1974**, *49*, 251.
(28) Sugawara, Y.; Hamada, Y.; Hifakawa, A. Y.; Tsuboi, M.; Kato, S.; Morokuma, K. *Chem. Phys.* **1980**, *50*, 105.
(29) Wright, G. M.; Simmonds, R. J.; Parry, D. E. *J. Comput. Chem.* **1988**, *9*, 601.
(30) Kwiatkowski, J. S.; Leszczyński, J. *J. Mol. Struct.* **1993**, *279*, 277.
(31) Caron, A.; Donohue, J. *Acta Crystallogr.* **1964**, *17*, 544.
(32) Caron, A.; Donohue, J. *Acta Crystallogr.* **1969**, *B25*, 404.
(33) Guth, H.; Heger, G.; Klein, S.; Treutmann, W.; Scheringer, C. *Z. Kristallogr.* **1980**, *153*, 237.
(34) Swaminathan, S.; Craven, B. M.; McMullan, R. K. *Acta Crystallogr.* **1984**, *B40*, 398.
(35) Kontoyianni, M.; Bowen, J. P. *J. Comput. Chem.* **1992**, *13*, 657.
(36) Rasul, G.; Prakash, G. K. S.; Olah, G. A. *J. Org. Chem.* **1994**, *59*, 2552.
(37) Šponer, J.; Hobza, P. *J. Phys. Chem.* **1994**, *98*, 3161.
(38) Head-Gordon, T.; Head-Gordon, M.; Frisch, M. J.; Brooks, C. L.; Pople, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 5989.
(39) Alex, A.; Clark, T. *J. Comput. Chem.* **1992**, *13*, 704.

Methods and Computational Details

All *ab initio* geometry optimizations at the RMP2-FC and RHF levels employed the standard 3-21G, 6-31G*, and 6-31G** basis sets[40] in the Gaussian 92 package.[41] Semiempirical AM1 geometry optimizations used the VAMP 4.40 program.[42] The computation of the chemical shifts with the standard 6-311G, 6-311G*, and 6-311G** basis sets employed the TX90-program (GIAO-SCF)[17] and ACES II (GIAO-MP2).[43-45] We used the H^t−N−C°−O dihedral angle[46] as a convenient measure to define the degree of nonplanarity.

We employed the 6-311G, 6-311G*, and 6-311G** basis sets to evaluate the dependence of the C° chemical shifts on the degree of nonplanarity of the environment of the amide nitrogen at GIAO-MP2 and GIAO-SCF for RMP2-FC/6-31G**-optimized geometries of formamide.

For *N*-formylglycine amide ($\phi = -48°$, $\psi = -57°$) (**1**) (also called glycine dipeptide analog, GDA), we optimized α-helical and β-pleated sheetlike ($\phi = -139°$, $\psi = 135°$) geometries with the AM1, RHF/3-21G, RHF/6-31G*, and RMP2-FC/6-31G* methods, and for *N*-acetyl-*N'*-methylglycine amide (**2**) (often referred to as glycine dipeptide, GD), α-helical and β-pleated sheetlike AM1 geometries were obtained. We computed the absolute NMR chemical shieldings for all structures of **1** and **2** at GIAO-SCF/6-311G and GIAO-SCF/6-311G* and evaluated the effect of the methyl groups by comparing the chemical shifts for GDA and GD at the GIAO-SCF levels.

To obtain $\Delta\delta$ ^{13}C C° as a function of the nonplanarity of the amide environment, we evaluated the GIAO-SCF/6-311G* chemical shifts for seven AM1-optimized geometries for an α-helical conformation of GD (**2**), which had both H−N−C°−O dihedral angles constrained to values between 180° and 150°. The NMR chemical shifts for seven RHF/6-31G*-optimized structures of the smaller *N*-formyl-*N'*-methylglycine amide (**3**), which were also optimized with both H−N−C°−O dihedral angles constrained to values between 180° and 150°, were computed at the GIAO-SCF/6-311G and GIAO-MP2/6-311G levels.

The calculations were carried out on IBM RS-6000 workstations at the Center for Computational Quantum Chemistry, the University of Athens, GA, and on the YMP-Cray at the Landesrechenzentrum München, Germany.

Results and Discussion

I. Formamide. The NMR chemical shieldings of C° depend strongly on the degree of out-of-plane bending at the nitrogen. Figure 2 shows the dependence of the NMR chemical shift (GIAO-MP2/6-311G**) of the carbonyl carbon (C°) on the nonplanarity of the amide nitrogen environment. Table 1 summarizes this dependence for C° with different basis sets at GIAO-MP2 and GIAO-SCF. For H^t−N−C°−O dihedral angles smaller than 170°, a nearly linear relationship between H^t−N−C°−O and the change in the chemical shielding of C° is found. Each 10° increment of the dihedral angle decreases the NMR chemical shielding by about 2 ppm. Table 2 gives the dependence of the NMR chemical shift of the formamide nitrogen on the out-of-plane bending of the amide nitrogen environment. The nitrogen is affected very little by the out-of-plane bending. The change in the relative chemical shielding is less than 1.5 ppm. The GIAO-SCF and GIAO-MP2 results

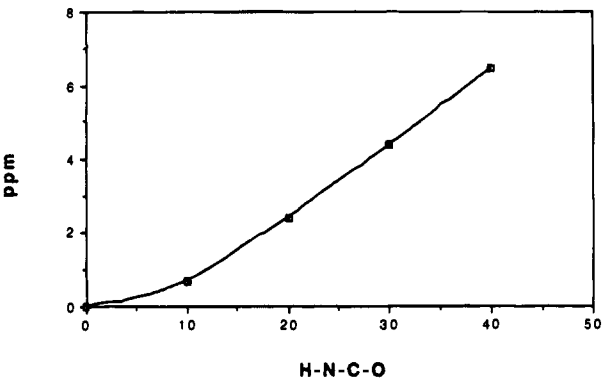


Figure 2. Dependence of the chemical shift of the carbonyl carbon in formamide on the nonplanarity of the amide nitrogen environment at the GIAO-MP2/6-311G**//MP2-FC/6-31G** level. The deviation of the H^t−N−C°−O dihedral angle from 180° was used as a measure of the degree of nonplanarity.

Table 1. Change in the NMR Chemical Shift (ppm) of C° in Formaldehyde as a Function of the H^t−N−C°−O Dihedral Angles[a]

	6-311G		6-311G*		6-311G**	
H^t−N−C°−O, deg	SCF[b]	MP2[c]	SCF[b]	MP2[c]	SCF[b]	MP2[c]
180	0.0	0.0	0.0	0.0	0.0	0.0
170	0.8	0.7	0.8	0.7	0.8	0.7
160	2.8	2.2	2.8	2.5	2.7	2.4
150	5.2	4.1	5.0	4.5	4.9	4.4
140	7.6	6.2	7.2	6.7	7.1	6.4

[a] The NMR chemical shift of H^t−N−C°−O = 180° was set equal to zero. All other values are relative to this. [b] GIAO-SCF. [c] GIAO-MP2.

Table 2. Change in the NMR Chemical Shift (ppm) of the Amide Nitrogen in Formaldehyde has a Function of the H^t−N−C°−O Dihedral Angle[a]

	6-311G		6-311G*		6-311G**	
H^t−N−C°−O, deg	SCF[b]	MP2[c]	SCF[b]	MP2[c]	SCF[b]	MP2[c]
180	0.0	0.0	0.0	0.0	0.0	0.0
170	0.3	0.3	0.0	0.1	0.0	0.0
160	1.0	1.1	0.1	0.5	0.0	0.3
150	1.2	1.7	−0.2	0.6	−0.4	0.3
140	0.8	1.8	−1.1	0.2	−1.4	−0.2

[a] The NMR chemical shift of H^t−N−C°−O = 180° was set equal to zero. All other values are relative to this. [b] GIAO-SCF. [c] GIAO-MP2.

are in excellent agreement. Obviously, the relatively small 6-311G basis set is sufficient to give a good description of the NMR chemical shielding.

II. Peptide Model Systems. A Ramachandran plot shows that only those backbone angles ϕ and ψ that correspond to structures with little steric hindrance are possible.[18] As a consequence, experimental NMR chemical shifts can only be obtained for conformations that correspond to certain values of ϕ and ψ. The two most common structural motifs are the α-helix and the β-pleated sheet. Two recent studies have evaluated a large number of experimental chemical shifts for those conformations.[20-22] Hence, we concentrated our investigation on those two backbone conformations and the difference in the NMR chemical shifts between them.

We compared geometries for an α-helical structure (**1a**)[47] and a β-pleated sheetlike structure (**1b**) of GDA, which were obtained with RMP2/6-31G*, RHF/6-31G*, RHF/3-21G, and

(40) Hehre, W. J.; Radom, L.; Pople, J. A.; Schleyer, P. v. R. *Ab Initio Molecular Orbital Theory*; John Wiley & Sons, Inc.: New York, 1986.
(41) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Ragahavachari, K.; Binkley, J. S.; Gonzales, G.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *GAUSSIAN 92*; Gaussian, Inc.: Pittsburgh, PA, 1992.
(42) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(43) The Aces II Program System: Stanton, J. F.; Gauss, J.; Watts, J. D.; Lauderdale, W. J.; Bartlett, R. *Int. J. Quantum Chem.* **1992**, *S26*, 879.
(44) Gauss, J. *Chem. Phys. Lett.* **1992**, *191*, 614.
(45) Gauss, J. *Chem. Phys.* **1993**, *99*, 3629.
(46) H^t denotes the hydrogen atom that is trans to the carbonyl oxygen.
(47) In the following, "a" always denotes an α-helical structure and "b" a β-pleated sheetlike structure.

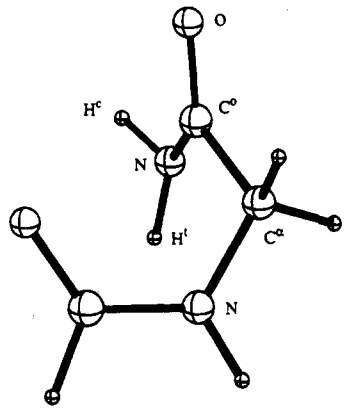
Table 3. $H^t-N-C^\circ-O$ and $H^c-N-C^\circ-O$ Dihedral Angles (deg) for the C-Terminal End of **1a** and **1b** at Various Levels of Theory

dihedral angle, deg	RMP2/6-31G*		RHF/6-31G*		RHF/3-21G		AM1	
	1a	**1b**	**1a**	**1b**	**1a**	**1b**	**1a**	**1b**
$H^t-N-C^\circ-O^a$	−158	174	−160	174	−175	176	−164	177
$H^c-N-C^\circ-O^b$	−21	0	−19	0	−8	−2	−14	0

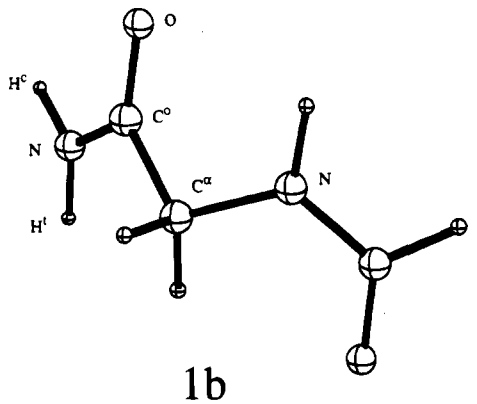
Table 4. Difference in the Relative Chemical Shifts (ppm) of C^α and C° at GIAO-SCF between an α-Helical Structure and a β-Pleated Sheetlike Structure of **1** Optimized at Various Levels of Theory

level at which the geometry was optimized	C^α		C°	
	6-311G	6-311G*	6-311G	6-311G*
RMP2-FC/6-31G*	6.3	6.7	−0.2	0.2
RHF/6-31G*	6.8	7.2	−1.3	−0.9
RHF/3-21G	5.9	6.4	−5.6	−4.5
AM1	6.7	7.1	−1.2	−1.0
experiment	2−5		2−5	

AM1. The differences in the bond lengths and the bond angles



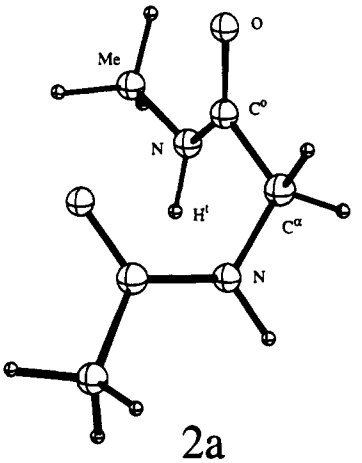
1a



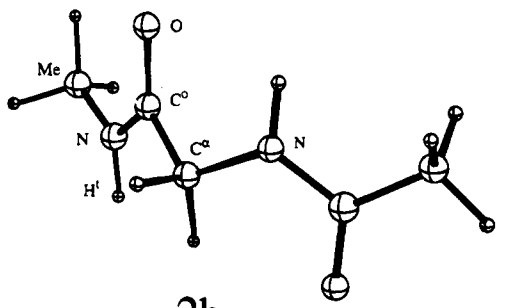
1b

are very small among these levels of theory. However, the $H^t-N-C^\circ-O$ and $H^c-N-C^\circ-O$ dihedral angles deviate considerably. The RMP2/6-31G*, RHF/6-31G*, and AM1 structures have partially pyramidalized nitrogen atoms in **1a**. The $H^t-N-C^\circ-O$ and $H^c-N-C^\circ-O$ dihedral angles deviate by 20° from the value that would be expected for a planar amide environment. However, at the RHF/3-21G level, both structure **1a** and **1b** have nearly planar nitrogen atoms (Table 3). The difference in the computed NMR chemical shifts of C° between an α-helical and a β-pleated sheetlike geometry demonstrates the influence of the pyramidalization of the nitrogen environment on peptide residues. Table 4 shows that the RMP2-FC/6-31G* geometry, which has the largest nonplanarity of the nitrogen environment for **1a**, is closest to the experimental range of 2−5 ppm. The discrepancy between experiment and theory is largest for the RHF/3-21G geometry, which predicts a largely planar amide nitrogen and has a $\Delta\delta$ ^{13}C C° of −5.6 (6-311G) and −4.5 ppm (6-311G*). A similarly poor result for δ ^{13}C C° (−4.6) ppm was obtained by Jiao *et al.* for *N*-acetyl-*N'*-methylglycine amide (**2**) at the IGLO/DZ//RHF/3-21G level.[3]

The AM1-optimized geometry of **2a** had a $H^t-N-C^\circ-O$ dihedral angle of −164.5°, and the AM1 geometry of **2b** had a $H^t-N-C^\circ-O$ dihedral angle of 177.5°. The $Me-N-C^\circ-C^\alpha$ dihedral angles (ω) were 174.2° (**2a**) and 178.4° (**2b**). Our



2a



2b

results for ω are in good agreement with those of Schaefer *et al.*, who have also found a deviation in ω of up to 6° in their *ab initio* RHF/4-31G study of *N*-formylpentaglycine amide and *N*-formylpentaalanine amide.[48] Moreover, Frey *et al.* have shown that the deviation of ω from 180° increases for *N*-formylalanine amide at correlated levels.[49] The differences in the NMR chemical shieldings between **2a** and **2b** were 5.3 ppm for C^α and 0.1 ppm for C° at the GIAO-SCF/6-311G level. At GIAO-SCF/6-311G*, the difference was 5.7 ppm for C^α and 0.2 ppm for C°. Hence, the addition of methyl groups reduces the difference in the chemical shifts for C^α by 1.4 ppm (GIAO-SCF/6-311G) and by 1.5 ppm (GIAO-SCF/6-311G*). The difference for C° is increased by 1.3 ppm (GIAO-SCF/6-311G) and by 1.2 ppm (GIAO-SCF/6-311G*). Therefore, while the dihedral angles are almost the same, the larger model system leads to an improvement of the agreement between theory and experiment.

Obviously, the amide moiety is nearly planar in a β-pleated sheetlike conformation but is partially pyramidal in an α-helical conformation. Figure 3 shows how the theoretical value for C° in **2** approaches the experimental range of 2−5 ppm as the degree of pyramidalization increases. The chemical shifts were

(48) Schaefer, L.; Newton, S. Q.; Cao, M.; Peeters, A.; Alsenoy, V. C.; Wolinski, K.; Momany, F. A. *J. Am. Chem. Soc.* **1993**, *115*, 272.

(49) Frey, R. F.; Coffin, J.; Newton, S. Q.; Ramek, M.; Cheng, V. K. W.; Momany, F. A.; Schaefer, L. *J. Am. Chem. Soc.* **1992**, *114*, 5369.

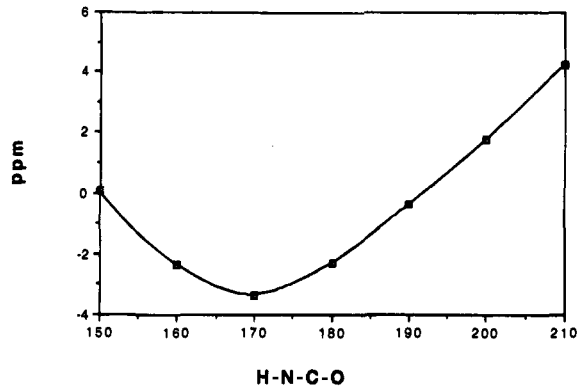


Figure 3. $\Delta\delta$ ^{13}C C° between several α-helical structures of *N*-acetyl-*N'*-methylglycine amide (**2**) and a β-pleated sheetlike structure of **2**. The α-helical structures had both H^t−N−C°−O dihedral angles constrained to obtain a nonplanar nitrogen environment.

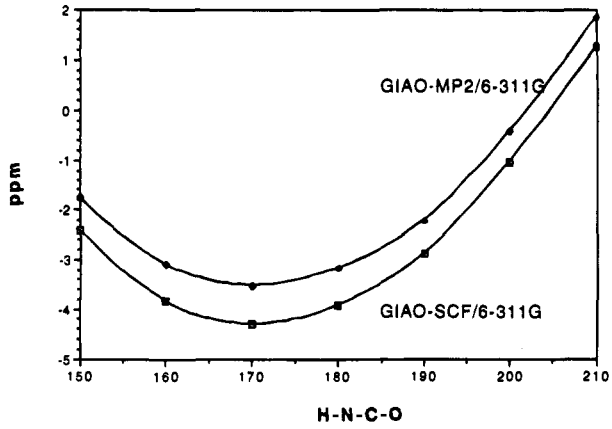


Figure 4. Dependence of the difference in the chemical shifts for C° in **3** between an α-helical structure and a β-pleated sheetlike structure on the degree of nonplanarity of the amide environment. The H^t−N−C°−O dihedral angle was used as measure of the nonplanarity.

computed at GIAO-SCF/6-311G*//AM1, and the H^t−N−C°−O dihedral angle was used as a measure of nonplanarity. Both H^t−N−C°−O dihedral angles of the α-helical conformation were constrained to the same value, and all other parameters except the backbone dihedrals were optimized. The value of $\Delta\delta$ ^{13}C C° is the difference between the NMR chemical shifts of the various α-helical structures and the δ C° result that was computed for a β-pleated sheetlike conformation.

For the smaller *N*-formyl-*N'*-methylglycine amide at GIAO-MP2/6-311G and at GIAO-SCF/6-311G, Figure 4 shows that, as the degree of pyramidalization increases, the computed $\Delta\delta$ ^{13}C C° approaches the experimental range of 2−5 ppm in agreement with our formamide results. *N*-Formyl-*N'*-methylglycine amide is the largest model system for which we can compute the NMR chemical shifts at the GIAO-MP2/6-311G level due to the large computational demands of the current implementation of the GIAO-MP2 module in ACES2.[45] For this model system, the C-terminal C$^\alpha$−C°−N−Me and the N-terminal H$_{C=O}$−C°−N−C$^\alpha$ dihedral angles were constrained to 180° to mimic real peptides, which are believed to have ω nearly 180°. Again, with increasing nonplanarity of the nitrogen environment, the computed $\Delta\delta$ ^{13}C C° approaches the experimental range of 2−5 ppm in agreement with the results for **2**. The dependence of $\Delta\delta$ ^{13}C C° on H^t−N−C°−O is smaller than for **2**, because ω was constrained to 180°.

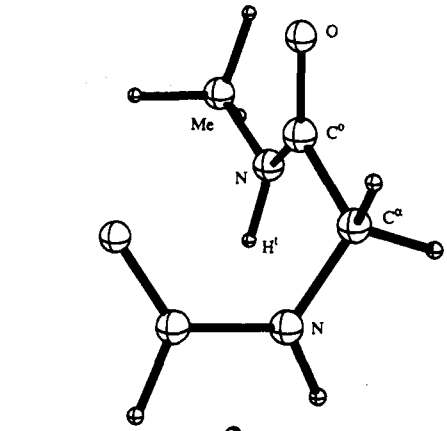
The NMR chemical shifts, which were obtained for peptide models with nearly planar nitrogen environments, reproduced the experimental $\Delta\delta$ ^{13}C of C$^\alpha$ and $\Delta\delta$ ^{15}N N_{amide} values

Table 5. Change in the Chemical Shieldings[a] for C$^\alpha$ and N as a Function of the Nonplanarity of the Amide Nitrogen Environment in **3a**

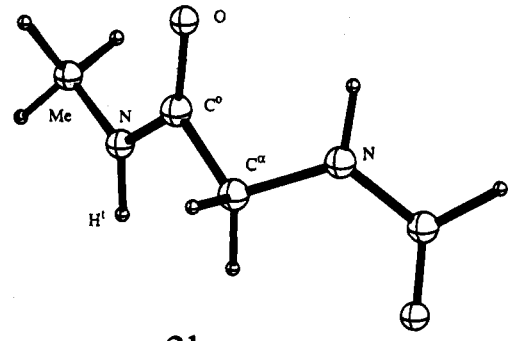
	C$^\alpha$		N	
H^t−N−C°−O, deg	GIAO-SCF[b]	GIAO-MP2[b]	GIAO-SCF[b]	GIAO-MP2[b]
150	6.7	9.3	−8.3	−8.0
160	6.3	8.8	−8.5	−8.7
170	6.0	8.4	−9.4	−10.2
180	5.9	8.2	−10.1	−11.2
−170	6.1	8.4	−9.7	−10.9
−160	6.4	8.6	−9.8	−10.8
−150	6.9	9.1	−10.0	−10.9

[a] The difference between the NMR chemical shift for an α-helical conformation with constraint H^t−N−C°−O dihedral angles and the chemical shift of a reference β-pleated sheetlike conformation is given. [b] The 6-311G basis set was employed.

between an α-helical structure and a β-pleated sheetlike structure.[3,50] Therefore, the NMR chemical shifts of C$^\alpha$ and N_{amide} should be only slightly affected by the nonplanarity of the nitrogen environment. Table 5 shows how the difference in the chemical shifts for C$^\alpha$ and N between conformation **3a** and **3b** depends on the degree of nonplanarity of the nitrogen environment. The chemical shifts were computed for the RHF/6-31G*-optimized geometries of **3** that we employed to obtain $\Delta\delta$ ^{13}C C°.



3a



3b

III. α-Helix Model. AM1 optimization of the parameters involving the hydrogen atoms in an α-helix model consisting of eight glycine residues gives an average H^t−N−C°−O dihedral angle of −164°. The Sybyl[51] program was used to build the backbone of the α-helix model. The atomic positions of the carbon, nitrogen, and oxygen atoms in an α-helix are well-known from X-ray studies, and this information was used

(50) Le, H. B.; Oldfield, E. *J. Biomol. NMR* **1994**, *4*, 341.
(51) Available from Tripos Associates, 1699 S. Hanley Road, Suite 303, St. Louis, MO 63144.

Table 6. Comparison of Experimentally Determined Hydrogen Bonding Parameters with the Results Obtained for Our Model α-Helix

	this study[a]	Phillips[b]	Taylor *et al.*[c]	Baker *et al.*[d]	Teeter *et al.*[d]
N−H (Å)	0.99		1.03	1.0[e]	
H···O (Å)	1.98	2.04	1.934	2.06(16)	
N···O (Å)	2.83			2.99(14)	2.85
N−H···O (deg)	142.9	156(13)	161.2(3)	155(11)	154.2
H···O=C°	148.7	(150)[f]		147(9)	147.9

[a] The average of the parameters obtained from residues 5−7 was used. [b] Neutron diffraction data.[53] [c] From a statistical analysis of neutron diffraction and X-ray data for organic crystals.[55−57] [d] From X-ray structures.[58,59] [e] A value of 1.0 Å was used as a constraint to obtain the other parameters. [f] Approximated from a figure.

for the parametrization of the Sybyl program. An average of the hydrogen bonding parameters between residues 2−5, 3−6, and 4−7 was used for the comparison with experimental data.

The N−H bond length, the N···O distance, the H···O hydrogen bond length, the N−H···O angle (donor angle), and the H···O−C° angle (acceptor angle) of the α-helix model are all in good agreement with experimental data obtained for analogous molecules with similar interactions.[52−59] Table 6 compares the parameters obtained from our model system with literature data. The donor angle deviates by 12−15° from the experimental average. However, a large number of N−H···O bonds are found for any value of the donor angle between 140 and 180°.[56] Also, a recent *ab initio* study revealed that in the 140−180° region of the N−H···O angle, the interaction energy is nearly constant at the HF/6-31G** level of theory.[60] Hence, a donor angle of 143° is in good agreement with experimental data.

The hydrogen positions cannot be refined in low-resolution X-ray structures. Instead, they are obtained by force field computations. High-resolution X-ray studies include hydrogen parameters only in the last steps of the refinement, but the hydrogen positions are not accurate either. Hence, we consider our value of −164° for the H^t−N−C°−O dihedral angle in an α-helix more trustworthy than previous experimental data.

Conclusions

Our exploration of the dependence of the NMR chemical shifts on the degree of nonplanarity of the nitrogen environment leads to the following conclusions. (a) The C° NMR chemical shift depends strongly on the pyramidalization of the amide moiety. (b) The difference in the NMR chemical shifts between an α-helical conformation and a β-pleated sheet for C° can be given correctly if a non-planar nitrogen environment with a H^t−N−C°−O dihedral angle of about −160° is assumed for an α-helix and a planar environment is assumed for a β-pleated sheetlike structure. However, in this study we excluded solvation effects under the assumption that the carbonyl carbon in both the α-helix and the β-pleated sheet forms roughly the same number of hydrogen bonds. This might not always hold. Chestnut *et al.* have computed that for glycylglycine, the effect of hydration is only in the range of 2−4 ppm. Nevertheless, solvation might be of importance. Furthermore, we did not take into account the fact that in solution many α-helices change from the classical structure with $\phi \approx -48°$ and $\psi \approx -57°$ to a more elongated conformation with $\phi \approx -62°$ and $\psi \approx -41°$ to make additional hydrogen bonding of the carbonyl carbon with a solvent molecule possible. (c) GIAO-MP2 and GIAO-SCF computations give almost the same results. (d) NMR chemical shifts computed with basis sets larger than the standard 6-311G basis lead only to small improvements in $\Delta\delta$. (e) As long as the H^t−N−C°−O dihedral angle is between 180° and −150°, the NMR chemical shielding of the nitrogen atom and of C^α are almost unaffected by changes in the nitrogen environment.

If the chemical shifts are evaluated for RMP2/6-31G*-, RHF/6-31G*-, and AM1-optimized geometries, which predict a non-planar amide nitrogen environment in an α-helix, $\Delta\delta$ ^{13}C C° deviates by only 1−3 ppm from the experimental range. Geometries for an α-helical structure that are computed at the RHF/3-21G level of theory, however, result in an almost planar amide nitrogen for the α-helical and the β-pleated sheetlike structure [H^t−N−C°−O = −175° (α-helix) and 176° (β-pleated sheet)]. The corresponding $\Delta\delta$ ^{13}C C° that is obtained for these geometries deviates from the experimental value by about 8 ppm. Geometries obtained at RHF/3-21G are therefore not appropriate for chemical shift computations of C°. Geometries obtained with empirical force fields are inappropriate because these also favor planar nitrogen environments.

A theoretical investigation of an α-helix model system consisting of glycine residues with the AM1 method gives an H^t−N−C°−O dihedral angle of about −164° in agreement with the results we obtained from *ab initio* studies of the smaller dipeptide model systems.

Acknowledgment. The work in Erlangen was supported by the Deutsche Forschungsgemeinschaft, the Fonds der Deutschen Chemischen Industrie (fellowship for H.M.S.), and the Convex Computer Corp. The work in Georgia was supported by the U.S. National Science Foundation (Grant CHE-9216754).

JA941772W

(52) *The Hydrogen Bond*; Schuster, P., Zundel, G., Sandorfy, C., Eds.; North-Holland Publishing Co.: Amsterdam, 1976.

(53) Phillips, S. E. V. Hydrogen Bonding and Exchange in Oxymyoglobin. In *Neutrons in Biology*; Schoenborn, B. P., Ed.; Plenum Press: New York, 1984.

(54) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin, 1991.

(55) Taylor, R.; Kennard, O.; Versichel, W. *J. Am. Chem. Soc.* **1983**, *105*, 5761.

(56) Taylor, R.; Kennard, O.; Versichel, W. *Acta Crystallogr.* **1984**, *B40*, 280.

(57) Taylor, R.; Kennard, O. *Acc. Chem. Res.* **1984**, *17*, 320.

(58) Baker, E. N.; Hubbard, R. E. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97.

(59) Teeter, M. M.; Roe, S. M.; Heo, N. H. *J. Mol. Biol.* **1993**, *230*, 292.

(60) Novoa, J. J.; Whangbo, M.-H. *J. Am. Chem. Soc.* **1991**, *113*, 9017.