Interrelationship between Conformation and Theoretical Chemical Shifts. Case Study on Glycine and Glycine Amide

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Abstract: The gauge independent atomic orbital (GIAO) method was used to explore the change in the chemical shift ($\Delta\delta$) with respect to the dihedral angle for glycine, H_2NCH_2COOH (**1**), and glycine amide, $H_2NCH_2CONH_2$ (**2**), at the GIAO-SCF/6-311G**//RHF/6-31G*, GIAO-SCF/6-311G**//MP2/6-31G*, and GIAO-MP2/6-311G**//MP2/6-31G* levels of theory. The absolute chemical shielding depends strongly on the geometry and the level of theory at which the NMR chemical shielding is calculated. The change of the chemical shifts as a function of the backbone angle ψ, however, depends only slightly on the level of theory. Both **1** and **2** are able to reproduce the difference in the chemical shifts for the methylene carbon (C_α) that is found for the α-helix and the β-pleated sheet. The chemical shifts of the diastereotopic hydrogens, that are bound to C_α, depend strongly on the positions of the carboxyl and carbamide moieties.

Introduction

The solution structure of a protein or peptide is in many cases identical to the biologically active structure that is found *in vivo*, because living cells exist in an aqueous environment.[2] Until about 15 years ago, X-ray crystallography was the only method to obtain structural information. Moreover, as discussed in detail by Kessler for cyclic peptides,[3] the structure in the crystal can differ considerably from that in solution for biomolecules. The structures of peptides in solution are now obtained by NMR methods including two- and three-dimensional Fourier-transform (FT) NMR experiments.[4] In a typical structure determination the NMR signals are assigned first. The nuclear Overhauser enhancement (NOE) effects between protons that are located at a shorter distance than approximately 5.0 Å from each other but belong to different parts of the amino acid sequence are determined next. In a possible structure of the peptide the above pairs of protons must be closer than 5 Å to each other. Distance–geometry algorithms, which use relatively simple force fields, perform a restricted geometry optimization to find possible spatial structures that contain the required HH distances.[4] Finally, using the Karplus equation, dihedral angles are deduced from vicinal coupling constants (^3J).[5,6]

NMR chemical shift information is not normally used to help deduce structural information, because the dependency of the NMR chemical shifts on the conformation is not well-known. Only in specific cases, e.g. to determine if a proline residue contains a cis or trans amide linkage, are chemical shifts employed.[7,8]

To establish this conformation–NMR shift relationship, two experimental approaches have been taken. The first is to measure the solid-state NMR shifts of proteins, whose X-ray structures are available.[9–11] The second is to assume that the crystal structure is maintained if the protein is dissolved and measure the NMR spectrum in solution.[12] Both methods provide information on the dependence of the NMR chemical shieldings on geometrical changes. However, the chemical shifts also depend on the side-chain conformation and crystal-packing effects, which complicate the analysis of the factors that influence δ.[9] If the NMR is taken in solution, the structure is not known well enough to determine structure–shift dependencies reliably. The problem is that small structural changes cannot be excluded. Finally, an experimental correlation between the chemical shifts and the conformation is limited to those secondary, or backbone, conformations that are represented in the actual molecule.

An alternative approach is to compute the dependencies of the NMR chemical shifts on the conformation using *ab initio* theory. Methods like IGLO,[13–17] LORG,[18–20] GIAO,[21–25] and GIAO-MP2[26,27] allow the prediction of the NMR chemical shifts and have been used successfully to elucidate the structures of boranes and carboranes,[28–31] have helped to reconfirm unusual NMR shifts,[32] and have elucidated long known NMR effects on a theoretical basis.[33] Furthermore, the absolute NMR chemical

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(1) (a) University of Georgia. (b) Universität Erlangen-Nürnberg.
(2) Streyer, L. *Biochemistry*; W. H. Freeman: New York, 1988.
(3) Kessler, H. *Angew. Chem.* **1982**, *94*, 509.
(4) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
(5) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11.
(6) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.
(7) McFarlane, W. *Chem. Commun.* **1970**, 418.
(8) Smith, I. C. P. In *Topics in Carbon-13 NMR-Spectroscopy*; Levy, G. C., Ed.; Wiley: New York, 1976; pp 1–80.
(9) Saitô, H. *Magn. Reson. Chem.* **1986**, *24*, 835.
(10) Teng, Q.; Iqbal, M.; Cross, T. A. *J. Am. Chem. Soc.* **1992**, *114*, 5312.
(11) Gerald, R.; Bernhard, T.; Haeberlen, U.; Rendell, J.; Opella, S. *J. Am. Chem. Soc.* **1993**, *115*, 777.
(12) Williamson, M. P. *Biopolymers* **1990**, *29*, 1423.
(13) Kutzelnigg, W. *Isr. J. Chem.* **1980**, *19*, 193.
(14) Schindler, M.; Kutzelnigg, W. *J. Chem. Phys.* **1982**, *76*, 1919.
(15) Schindler, M.; Kutzelnigg, W. *J. Am. Chem. Soc.* **1983**, *105*, 1360.
(16) Fleischer, U.; Schindler, M. *Chem. Phys.* **1988**, *120*, 103.
(17) Kutzelnigg, W.; Fleischer, U.; Schindler, M. *NMR Basic Principles and Progress*; Springer-Verlag: Berlin Heidelberg, 1990; Vol. 23.
(18) Hansen, A. E.; Bouman, T. D. *J. Chem. Phys.* **1985**, *82*, 5035.
(19) Bouman, T. D.; Hansen, A. E. *Chem. Phys. Lett.* **1988**, *149*, 510.
(20) Hansen, A. E.; Bouman, T. D. *J. Chem. Phys.* **1989**, *91*, 3552.
(21) Ditchfield, R. *J. Chem. Phys.* **1972**, *56*, 5688.
(22) Ditchfield, R. *MTP International Review of Science Physical Chemistry Series 1. Molecular Structure and Properties*; Butterworth: London, 1972.
(23) Ditchfield, R. *Mol. Phys.* **1974**, *27*, 789.
(24) Ditchfield, R.; Ellis, P. D. In *Topics in Carbon-13 NMR Spectroscopy*; Levy, G. C., Ed.; Wiley: New York, 1974.
(25) Pulay, P.; Wolinski, K.; Hinton, J. F. *J. Am. Chem. Soc.* **1990**, *112*, 8251.
(26) Gauss, J. *Chem. Phys. Lett.* **1992**, *191*, 614.
(27) Gauss, J. *Chem. Phys.* **1993**, *99*, 3629.
(28) Hnyk, D.; Vajda, E.; Bühl, M.; Schleyer, P. v. R. *Inorg. Chem.* **1992**, *31*, 2464.
(29) Bühl, M.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1992**, *114*, 477.
(30) Bühl, M.; Schleyer, P. v. R. *Struct. Chem.* **1993**, *4*, 1.
(31) Mebel, A. M.; Charkin, O. P.; Schleyer, P. v. R. *Inorg. Chem.* **1993**, *32*, 469.

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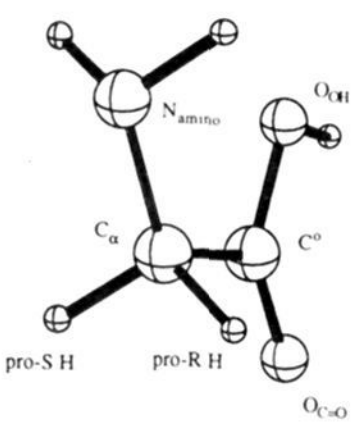
shieldings have been computed for a large number of molecules.[17] In general, the relative chemical shifts that are obtained show good agreement with experimental data.

Chemical shifts have already been computed *ab initio* for peptide structures. De Dios, Pearson, and Oldfield found good agreement between GIAO NMR chemical shifts and the experimental values for the C_α- and C_β-carbons as well as for nitrogen (^{15}N) and fluorine (^{19}F) atoms of selected residues in protein models.[34] In addition, they claim that the various parameters, which affect the chemical shifts (bond length, bond angle, and dihedral angle) are not coupled and can therefore be treated separately.[35] Laws et al. reproduced the chemical shifts of the C_α-carbon using the GIAO method.[36] Jiao, Barfield, and Hruby showed that IGLO calculations can reproduce the shift difference between the syn- and anti-conformation for the *N*-methyl group in simple amides.[37] They also used the IGLO program to obtain the chemical shielding surface for C_α of *N*-acetyl-*N'*-methylglycinamide and are able to reproduce the experimental values for Cα but not for C^O.[38] Chestnut and Phung pointed out that the NMR chemical shieldings of glycine dipeptide, computed with the GIAO method, are affected by intra- and intermolecular hydrogen bonding. Furthermore, they showed that an NMR-shift computation that uses large basis sets for the atoms of interest and small basis sets for all other atoms (the "locally dense" approach) can give acceptable results.[39-41] The molecular geometries that are used in the NMR-shift calculations may be obtained either from *ab initio* calculations[37-40,42-45] or *from crystal structures.*[35]

We chose the simplest amino acid, glycine (**1**), as well as glycine amide (**2**), because it contains the amide moiety found in all peptides for this study. We have evaluated the dependence of the NMR chemical shieldings on the conformations of **1** and **2**



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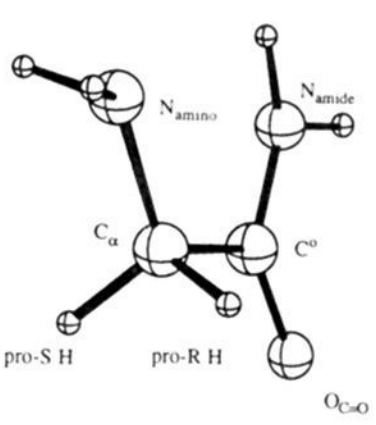

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employing both GIAO-SCF and GIAO-MP2. The usefulness of such knowledge for conformational assignments was shown by

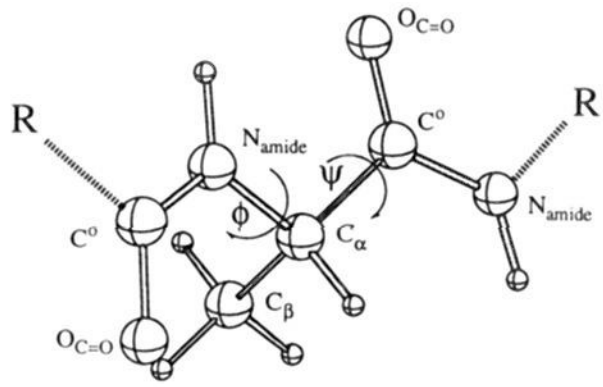


Figure 1. Definition of the backbone angles ϕ and ψ, shown for an alanine residue ($\phi = \psi = 180°$). The dihedral angle C^O–N–C_α–C^O is designated ϕ, and the dihedral angle N–C_α–C^O–N is designated ψ. The eclipsed conformer has $\phi = 0°$ and $\psi = 0°$. Rotation to the right is counted positive.

Buchanan, Morat, and Kirby.[46] Their GIAO computations of ethylene glycol revealed that δ ^{13}C changes over a range of 8 ppm when the torsional angle between the two carbon atoms is changed in 20-deg increments going from 0° to 180°. Comparison with experimental NMR data confirmed their structural assignment for an 18-crown-6 ether derivative.

The backbone conformation of peptides is defined by the angles ψ and φ. The dihedral angle N–C_α–C^O–N is called ψ, and the dihedral angle C^O–N–C_α–C^O is called ϕ.[47] By definition, the eclipsed conformer has $\psi = 0°$ and $\phi = 0°$. Rotation to the right is counted positive. Figure 1 shows these definitions for an alanine residue. For both ψ and φ, several values exist that correspond to known minima structures for peptides. These define allowed regions in a Ramachandran plot.[47] For **1** and **2** the torsional angle, ψ, which we used as the reaction coordinate, was defined as the dihedral angle N–C_α–C^O–O_{OH} for **1** and N–C_α–C^O–N for **2**, in analogy to the backbone angle ψ in peptides.

The NMR chemical shieldings of **1** and **2** were evaluated at the SCF level of theory with the GIAO algorithm as implemented in TX90 for each increment of ψ of 10°. In addition, GIAO-MP2 shifts were computed with the ACES 2 program[48] for the MP2/6-31G*-optimized structures of both molecules with ψ = 0°, 60°, and 120°. Since only the effect of ψ was investigated, the influence of ϕ on the NMR chemical shifts is not known. Moreover, since the amino nitrogen in **1** and **2** is sp^3-hybridized and not planar as in a peptide, some additional influence, that is not found in proteins, cannot be excluded. However, our results indicate that the shifts of atoms that do not belong to the amino group are only moderately affected by changes in the orientation of the amino hydrogens.

The questions we are interested in answering are (1) What is the magnitude of the changes in chemical shielding for the various atoms in our model compounds? (2) Is it sufficient to calculate the chemical shifts at the SCF level of theory, or do correlated methods (GIAO-MP2) give different results? (3) What level of theory should be used for the geometry optimizations? (4) Will the structures of our simple molecules with a dihedral angle ψ that corresponds to a α-helix or a β-pleated sheet show the same difference in the chemical shielding as determined experimentally for peptides?[49,50] (5) To what extent does the relative position of the carboxyl or carbamide group influence the relative chemical shielding and the difference in the NMR chemical shifts between the diastereotopic protons at the methylene carbon of the glycine moiety? This is of interest, because a correlation between the

(32) Quast, H.; Ach, M.; Kindermann, M. K.; Rademacher, P.; Schindler, M. *Chem. Ber.* **1993**, *126*, 503.
(33) Imashiro, F.; Masuda, Y.; Honda, M.; Obara, S. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1535.
(34) De Dios, A. C.; Pearson, J. G.; Oldfield, E. *Science* **1993**, *260*, 1491.
(35) De Dios, A. C.; Pearson, J. G.; Oldfield, E. *J. Am. Chem. Soc.* **1993**, *115*, 9769.
(36) Laws, D. D.; de Dios, A. C.; Oldfield, E. *J. Biomol. NMR* **1993**, *3*, 607.
(37) Jiao, D.; Barfield, M.; Hruby, V. *Magn. Reson. Chem.* **1993**, *31*, 75.
(38) Jiao, D.; Barfield, M.; Hruby, V. J. *J. Am. Chem. Soc.* **1993**, *115*, 10883.
(39) Chestnut, D. B.; Phung, C. G. *Chem. Phys. Lett.* **1991**, *183*, 505.
(40) Chestnut, D. B.; Phung, C. G. In *Nuclear Magnetic Shieldings and Molecular Structure*; Tossell, J. A., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1993.
(41) Chestnut, D. B.; Rusiloski, B. E.; Moore, K. D.; Egolf, D. A. *J. Comput. Chem.* **1993**, *11*, 1364.
(42) Schäfer, L.; Newton, S. Q.; Momany, F. A.; Klimkowski, V. J. *THEOCHEM* **1991**, *232*, 275.
(43) Böhm, H.-J.; Brode, S. *J. Am. Chem. Soc.* **1991**, *113*, 7129.
(44) Kollman, P. A.; Gould, I. R. *J. Phys. Chem.* **1992**, *96*, 9255.
(45) Schäfer, L.; Newton, S. Q.; Ming, C.; Peeters, A.; Van Alsenoy, C.; Wolinski, K.; Momany, F. A. *J. Am. Chem. Soc.* **1993**, *115*, 281.
(46) Buchanan, G. W.; Morat, C.; Kirby, R. A.; Tse, J. S. *Can. J. Chem.* **1991**, *69*, 1964.
(47) Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. *J. Mol. Biol.* **1963**, *7*, 95.
(48) Stanton, J. F.; Gauss, J.; Watts, D.; Lauderdale, W. J.; Bartlett, R. J. The ACES II Program System. *Int. J. Quantum Chem.* **1992**, *S26*, 879.
(49) Spera, S.; Bax, A. *J. Am. Chem. Soc.* **1991**, *113*, 5490.
(50) Reily, M. D.; Thanabal, V.; Omecinsky, D. O. *J. Am. Chem. Soc.* **1992**, *114*, 6251.

latter and the backbone structure exists, which allows a structural assignment.[51]

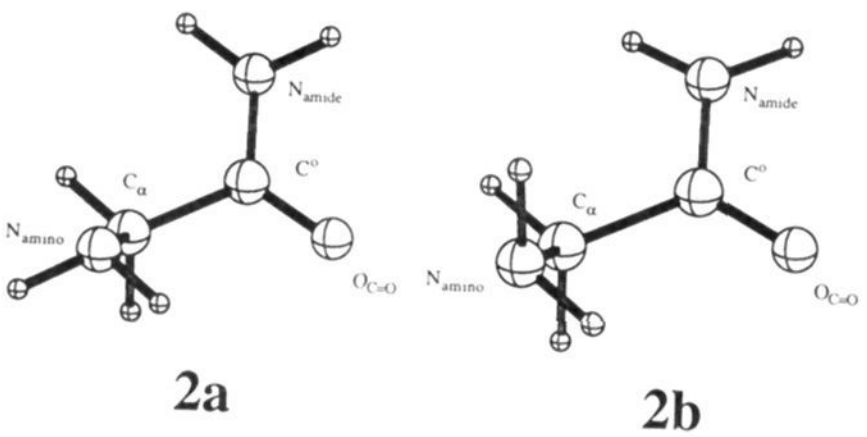
Computational Details

The geometries used in the NMR chemical shift studies were optimized using the self-consistent-field (SCF) analytic gradient method CADPAC 5.1.[52] Harmonic vibrational frequencies were computed with SCF analytic second-derivative methods. Saddle points were located using the Schlegel method.[53] For glycine (**1**) the dihedral angle NH_2–C_α–C^O–O_{OH} and for glycine amide (**2**) the dihedral NH_2–C_α–C^O–N_{amide} were used as the reaction coordinates. The potential energy surface, local minima, and transition states were computed fully. An RHF/6-31G* structure was minimized for each dihedral angle increment of 10°. In addition, a structure for $\psi = 0°$, 60°, and 120° was optimized at the MP2/6-31G* level with Gaussian 92.[54] The SCF NMR chemical shift calculations employed the TX90 program[25] with its implementation of the GIAO[21-24] method and the standard 6-311G** basis set.[55] The MP2 NMR shift calculations were performed with the ACESII program package[26,27,48] and employed the same basis set.

The calculations were carried out on IBM RS-6000 workstations at the Center for Computational Quantum Chemistry and an Indigo-Iris at the Department of Chemistry, University of Georgia, Athens. Geometry optimizations (RHF/6-31G*) took ca. 1–2 h, GIAO-SCF shift calculations 70 min, and GIAO-MP2 shift calculations 10 h each on an IBM RS-6000/580 workstation. For an IBM RS-6000/530 workstation these benchmarks must be multiplied by a factor of 2.5.

Results and Discussion

Geometries. In agreement with recent results,[56,57] the global minimum of glycine has C_s symmetry and $\psi = 180°$. A second minimum, at a torsional angle of 0°, has C_s symmetry and is 1.9 kcal/mol higher in energy. The global minimum for glycine amide is located at $\psi = 165°$. A second minimum, 1.3 kcal/mol higher in energy, was found at 2°. The structure with $\psi = 180°$ has C_1 symmetry and resembles a distorted version of the global minimum. The main difference is that for $\psi = 165°$ the two hydrogens bound to the amino nitrogen form hydrogen bonds of a different length with the carbonyl oxygen. Up to $\psi = 110°$ a structure with one amino hydrogen oriented anti-coplanar to the carbon–carbon bond is lowest in energy (**2a**). In the remaining structures both hydrogens form hydrogen bonds with the carbonyl oxygen (**2b**).



2a **2b**

NMR Chemical Shifts. Equation 1 was used whenever we were interested in the relative change of the chemical shifts, δ. We used the absolute shielding, σ, at $\psi = 0°$ as σ_{ref}. A more detailed explanation of the relationship between σ and δ is found elsewhere.[17,21]

$$\delta = \sigma_{ref} - \sigma \quad (1)$$

For the future application of NMR chemical shift calculations to proteins and peptides, it is important to know how the results depend on the quality of the geometry that is used. Even more so, the effects of electron correlation have to be elucidated. Hence, we have computed GIAO-SCF chemical shieldings, σ, for RHF/6-31G*- and RMP2/6-31G*-optimized geometries of **1** and **2** for $\psi = 0°$, 60°, and 120°. For the MP2 geometry σ was also computed with the GIAO-MP2/6-311G** method. For all atoms the absolute chemical shielding decreases, when an MP2-optimized geometry is used instead of an RHF-optimized one (Table 1). For $O_{C=O}$ the difference is up to 30 ppm (Figure 2), for N_{amide} and C^O, 8–12 ppm, and for C_α 2–3 ppm (Figure 3).

Table 1. Absolute Chemical Shielding for Some Atoms of Glycine (**1**) and Glycine Amide (**2**)[a]

angle ψ	method[b]	0°	60°	120°
		Glycine		
C_α	I	153.2	151.9	153.7
	II	150.6	149.9	151.4
	III	150.3	149.0	151.3
C^O	I	20.9	22.5	19.5
	II	9.5	10.9	7.4
	III	27.5	28.4	25.1
$O_{C=O}$	I	−57.7	−75.6	−54.5
	II	−88.2	−108.6	−85.9
	III	−49.9	−65.1	−46.3
		Glycine Amide		
C_α	I	153.0	149.8	150.9
	II	150.5	147.5	148.7
	III	150.1	146.0	148.3
C^O	I	20.0	18.7	18.3
	II	10.1	7.7	7.3
	III	30.5	27.9	26.9
$O_{C=O}$	I	−41.7	−87.2	−42.0
	II	−63.8	−114.0	−67.9
	III	−38.7	−81.8	−39.8
N_{amide}	I	179.6	180.6	176.9
	II	170.9	171.6	168.2
	III	179.8	179.8	177.5

[a] The absolute chemical shielding is very dependent on the geometry and electron correlation for all atoms except C_α. However, the relative change in the chemical shielding with respect to the torsional angle is almost the same for I, II, and III. This suggests that once the chemical shifts have been computed at a high level of theory for one conformation, the chemical shifts for other conformations can be obtained with less extensive computations. [b] (I) GIAO-SCF/6-311G**//RHF/6-31G*, (II) GIAO-SCF/6-311G**//MP2/6-31G*, and (III) GIAO-MP2/6-311G**//MP2/6-31G*.

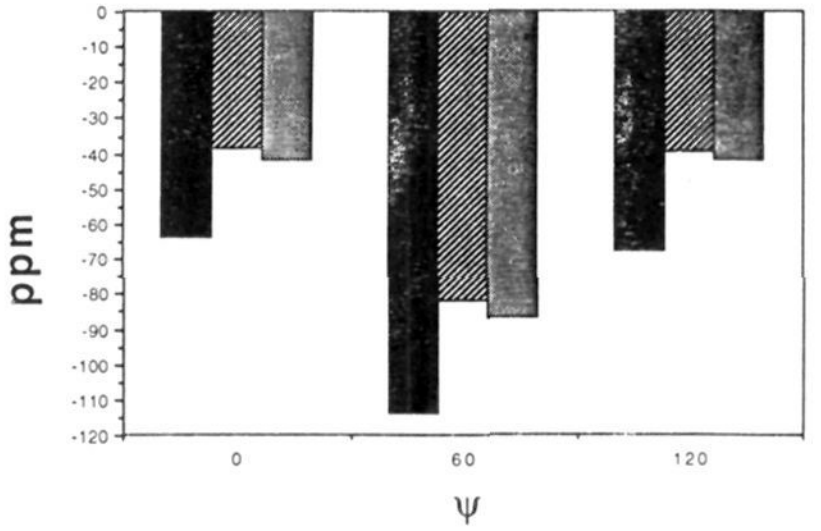


Figure 2. Absolute chemical shielding calculated for $O_{C=O}$ of glycine amide at the GIAO-SCF/6-311G**//MP2/6-31G* level (first bar), GIAO-MP2/6-311G**//MP2/6-31G* level (second bar), and GIAO-SCF/6-311G**//RHF/6-31G* level of theory (third bar) for 0°, 60°, and 120° torsion. (The first bar corresponds to the left-most bar in each set.)

(51) Williamson, M. P.; Asakura, T. *FEBS Lett.* **1992**, *302*, 185.
(52) CADPAC5: The Cambridge Analytic Derivatives Package Issue 5.1, Cambridge, 1993. A suite of quantum chemistry programs developed by R. D. Amos, N. C. Handy, and co-workers.
(53) Schlegel, H. B. *J. Comput. Chem.* **1982**, *3*, 214.
(54) 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.
(55) Hehre, W. J.; Random, L.; Pople, J. A.; Schleyer, P. v. R. *Ab Initio Molecular Orbital Theory*; John Wiley & Sons, Inc.: New York, 1986.
(56) Csaszar, A. G. *J. Am. Chem. Soc.* **1992**, *114*, 9568.
(57) Hu, C.-H.; Shen, M.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **1993**, *115*, 2923.

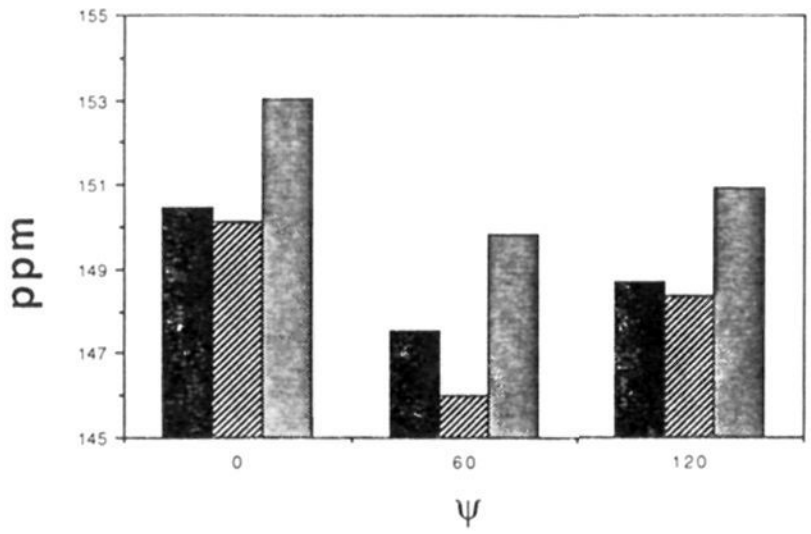


Figure 3. Absolute chemical shielding calculated for C_α of glycine amide at the GIAO-SCF/6-311G**//MP2/6-31G* level (first bar), GIAO-MP2/6-311G**//MP2/6-31G* level (second bar), and GIAO-SCF/6-311G**//RHF/6-31G* level of theory (third bar) for 0°, 60°, and 120° torsion. (The first bar corresponds to the left-most bar in each set.)

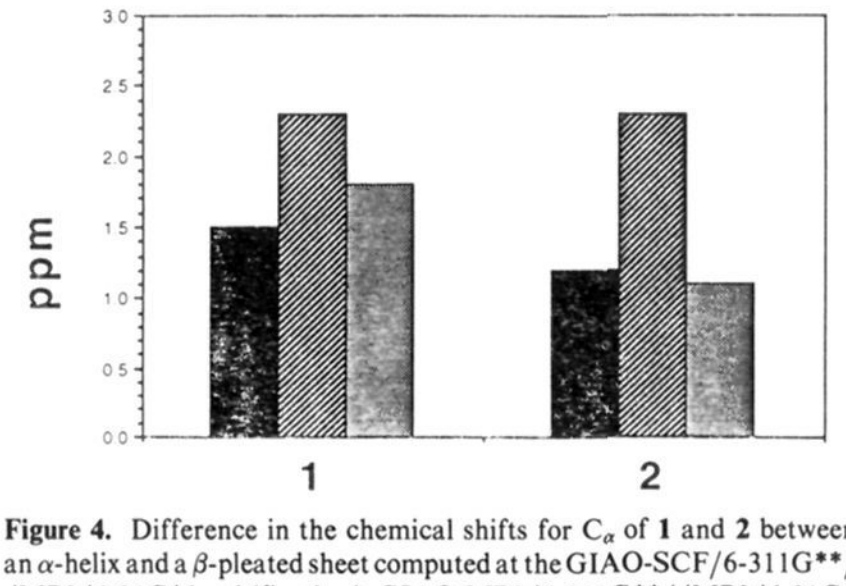


Figure 4. Difference in the chemical shifts for C_α of **1** and **2** between an α-helix and a β-pleated sheet computed at the GIAO-SCF/6-311G**//MP2/6-31G* level (first bar), GIAO-MP2/6-311G**//MP2/6-31G* level (second bar), and GIAO-SCF/6-311G**//RHF/6-31G* level (third bar). The numbers on the x-axis correspond to glycine and glycine amide. (The first bar corresponds to the left-most bar in each set.)

The values for the absolute chemical shielding σ, which are obtained with the GIAO-MP2 method, exceed the corresponding GIAO-SCF results for the same geometry by up to 30 ppm for $O_{C=O}$ (Figure 2) and 8–12 ppm for N_{amide}. For C^O they increase by ca. 20 ppm. For C_α only with $\psi = 60°$ a larger difference of 1–2 ppm is observed (Figure 3). Despite the sensitivity of the absolute chemical shielding on the geometry and correlation effects, the change of the absolute chemical shielding with respect to the torsional angle is qualitatively the same for all three computations (Table 1). Hence, it may be sufficient to compute GIAO-MP2 shifts for only one conformer and use the relative changes that are obtained at the SCF level to estimate the chemical shifts for other conformers. For C_α however, this procedure will not give very accurate results, because the difference between the GIAO-SCF and GIAO-MP2 results is not the same for conformers with different ψ. Therefore, in order to be able to predict the chemical shifts in proteins accurately, small model systems have to be explored first to determine the influence of electron correlation.

Studying a large number of peptide shifts, Spera and Bax have found that the α-carbons of amino acid residues in an α-helix ($\psi = -60°$) are shifted downfield by 4–5 ppm as compared to those in a β-pleated sheet ($\psi = 120°$).[49] In order to evaluate our simple model systems, we compared the difference between the chemical shifts of C_α and C^O [58] at $\psi = 60°$ (for glycine and glycine amide δ for –60° and 60° are the same, since they are not chiral) and

(58) For ^{17}O no comparison could be made, because no experimental data are available.

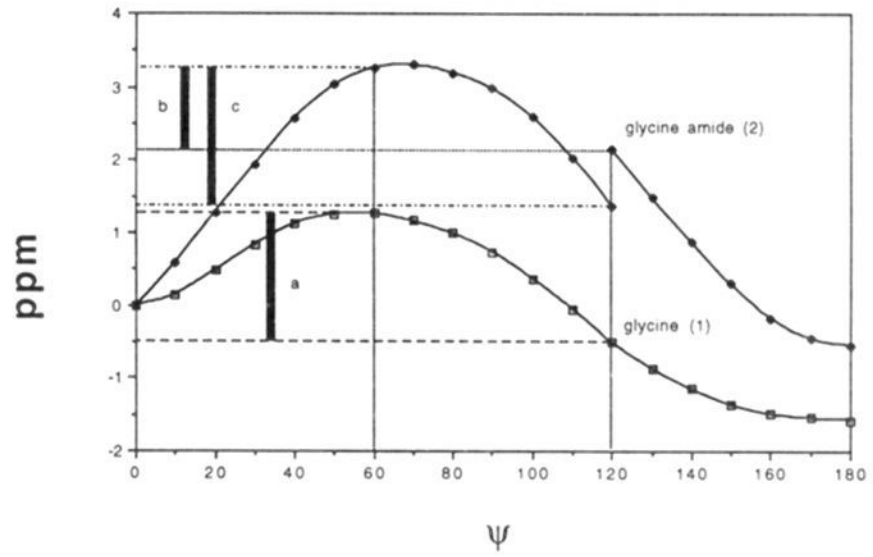


Figure 5. Change in the NMR chemical shifts of the α-carbons of **1** and **2** as a function of the torsional angle (ψ) at the GIAO-SCF/6-311G**//RHF/6-31G* level. The solid lines correspond to the shifts that are obtained for the α-helical- and β-pleated-sheet-like structures. For **1** the difference between both structures is 1.8 ppm (a). For **2** there are two possible values: 1.1 ppm (b) using structure **2b** at 120° or 1.9 ppm (c) with the second amino hydrogen still anti-coplanar to the carbon–carbon bond. We favor c, since it corresponds to a continuous curve.

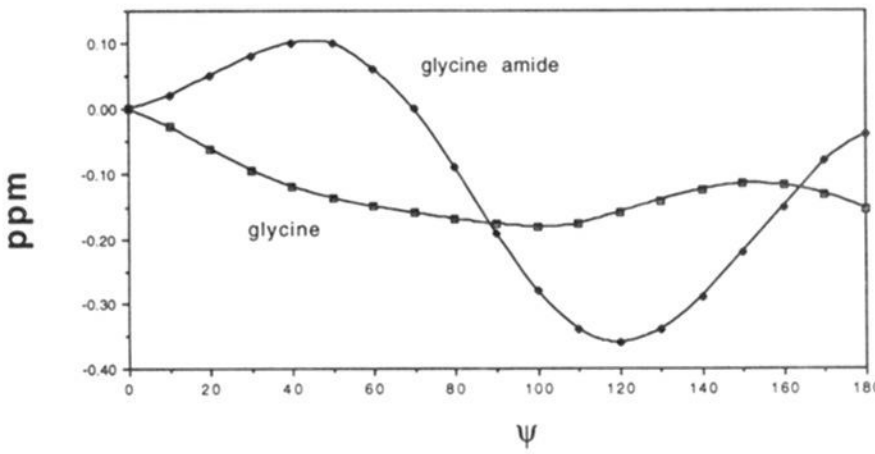


Figure 6. Change in the chemical shielding for the *pro-R*-hydrogen of **1** and **2** as a function of the torsional angle (ψ) at the GIAO-SCF/6-311G**//RHF/6-31G* level. The α-hydrogen of **2** shows a much stronger dependence on ψ than the α-hydrogen of **1**.

$\psi = 120°$ for **1** and **2**. For C_α the difference is between 1.5 and 2.3 ppm for **1** and between 1.1 and 2.3 ppm for **2** (Figure 4). GIAO-MP2 gives the largest difference, 2.3 ppm. In a peptide all nitrogen atoms of the backbone form amide linkages with the C^O of the neighboring residues (Figure 1). They have only one hydrogen left that can interact with the rest of the molecule. Therefore, a geometry for **2** with $\psi = 120°$, that still has one hydrogen pointing away from the rest of the molecule (similar to **2a**), mimics the peptide moiety more closely, although this geometry lies slightly higher in energy for $\psi = 120°$ than **2b**. For this structure the difference for **2** becomes 1.9 ppm at the GIAO-SCF/6-311G**//RHF/6-31G* level. Since, in addition, the 1.9 ppm value coresponds to a steady curve, we consider it to be more accurate. However, for C^O **1** and **2** do not reproduce the difference in the chemical shielding between an α-helix-like structure and a β-pleated-sheet-like structure correctly. Since the incorrect result is reproduced at all levels of theory, we believe that our model is too limited to reproduce the right shielding for C^O, because it does not account for the effect of a second carbonyl group whose relative position is determined by ϕ. Interaction of the two π-systems with each other should affect the chemical shielding at C^O. Another contributing factor may be the neglect of interresidue hydrogen bonding.

From a comparison with a plot of the NMR chemical shielding of C_α in *N*-acetyl-*N'*-methylglycinamide versus ψ and ϕ,[38] it can be seen that the change in the NMR shifts of C_α with respect to ψ does not depend on the value of ϕ. A plot of the chemical shift of C_α versus ψ has a very similar shape for all values of ϕ. We conclude that glycine and glycine amide allow a correct description

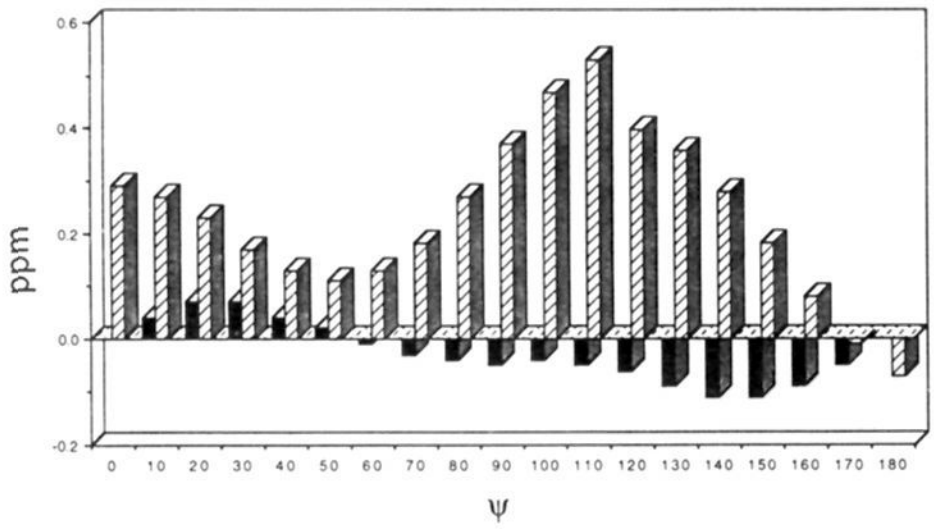


Figure 7. Change in the difference between the diastereotopic protons of glycine (black) and glycine amide (shaded) as a function of the torsional angle ψ at the GIAO-SCF/6-311G**/RHF/6-31G* level. The absolute chemical shielding of the *pro-S*-proton was subtracted from the absolute chemical shielding of the *pro-R*-proton. Since the structures of glycine amide at $\psi = 0°$ and $\psi = 180°$ do not have C_s symmetry, the NMR chemical shifts of the diastereotopic protons are not equal.

of the changes in the chemical shielding for the α-carbon of peptides due to changes in ψ. Since **1** and **2** both have no side chain, the size of the effect is smaller than for other amino acids. Figure 5 shows the change of the shift for the α-carbon over the full torsional range at the GIAO-SCF/6-311G**//RHF/6-31G* level of theory.

A large number of studies have investigated the change of the chemical shift(s) of the proton(s) at the α-carbon in peptides,[12,59–66] which we henceforth call α-protons. Of the two protons, the *pro-R*-proton is present in all amino acids, whereas only glycine has the *pro-S*-proton, which is replaced by the respective side chain in all other residues. All three computations show that for **1** the *pro-R*-proton is only very moderately (±0.2 ppm) affected by changes in ψ. The *pro-R*-proton of **2**, however, is very susceptible to changes in ψ (Figure 6). First it becomes slightly deshielded, and then, as the distance between the α-proton and that amide proton that points toward it decreases, it becomes increasingly shielded. The largest shielding is found for $\psi = 120°$, the geometry where the above-mentioned protons are closest in space. GIAO-MP2 reproduces the overall change. However, the deshielding at $\psi = 60°$ is larger (0.30 ppm) and the shielding at 120° is only –0.22 ppm. The difference in the chemical shielding for the *pro-R*-α-proton of **2** between the geometry with $\psi = 60°$ and 120° is –0.45 ppm (GIAO-SCF/6-311G**//RHF/6-31G*), –0.44 ppm (GIAO-SCF/6-311G**//MP2/6-31G*), and –0.52 ppm (GIAO-MP2/6-311G**//MP2/6-31G*). Compared to the 10 ppm spectral width for proton NMR, this is a large change. It has been shown by Williamson that the mean α-proton shift in helices is 0.65 ppm upfield of the mean α-proton shift in sheets.[12] For our calculations we find that for **2** the proton in an α-helix-like structure is shifted downfield by 0.37 ppm (GIAO-SCF/6-311G**//RHF/6-31G*), 0.41 ppm (GIAO-SCF/6-311G**//MP2/6-31G*), and 0.07 ppm (GIAO-MP2/6-311G**//MP2/6-31G*) compared to that in a β-pleated-sheet-like structure. The disagreement with the experimental results must be due to our limited model which does not account for ϕ. Nevertheless, our results show that electron correlation is important for the proton chemical shifts.

In most cases, programs that use empirical parameters derived from a large number of well-known structures to calculate NMR chemical shifts are parametrized only for σ^{ring} (ring current effects of neighboring groups), σ^{E} (effects due to intramolecular electric fields), and σ^{anti} (magnetic anisotropy effects).[65] However, taking into account the shape of the plot of the change in the NMR chemical shielding for the α-proton in the amide (Figure 6), we suggest that a term might be included that depends on the distance between the amide hydrogen and the α-hydrogen. To understand this effect better, we are studying larger systems.

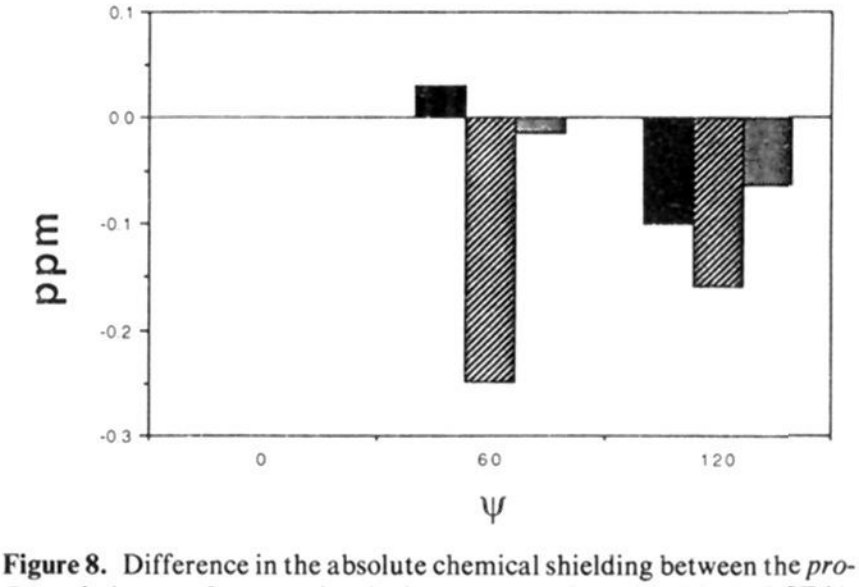


Figure 8. Difference in the absolute chemical shielding between the *pro-R*- and the *pro-S*-proton in glycine, computed at the GIAO-SCF/6-311G**//MP2/6-31G* level (first bar), GIAO-MP2/6-311G**//MP2/6-31G* level (second bar), and GIAO-SCF/6-311G**//RHF/6-31G* level of theory (third bar) for $\psi = 0°$, 60°, and 120°. (The first level corresponds to the left-most bar in each set.)

Williamson and Asakura have shown that the difference in the chemical shielding between the two diastereotopic α-protons can be used for conformational assignments.[51] At the SCF level our calculations gave a much larger difference for the diastereotopic protons in **2** than in **1** with a maximum for $\psi = 120°$. The GIAO-MP2 results show about the same difference for both molecules (Figures 7–9). The large difference between the SCF and MP2 results makes a reliable computation of the shifts of the α-hydrogens and their difference at the SCF level seem unlikely. However, once the effect of electron correlation, which is mostly caused by the carboxy or carbamide moiety, has been determined for the full torsional space, it should be possible to apply an empirical correction term to SCF results.

Conclusions

The absolute NMR chemical shielding depends strongly on the geometry and the level of theory at which the geometry and the NMR chemical shifts are computed. The changes range from 2–3 ppm for C_α to as much as 30 ppm for $O_{C=O}$. However, the relative change of the chemical shifts with respect to the dihedral angle ψ is almost the same at the GIAO-SCF/6-311G**/

(59) Asakura, T.; Ando, I.; Nishioka, A. *Makromol. Chem.* **1977**, *178*, 1111.
(60) Asakura, T.; Ando, I.; Nishioka, A. *Makromol. Chem.* **1977**, *178*, 1521.
(61) Asakura, T. *Makromol. Chem.* **1981**, *182*, 1135.
(62) Asakura, T. *Makromol. Chem.* **1981**, *182*, 1153.
(63) Asakura, T. *Makromol. Chem.* **1981**, *182*, 1109.
(64) Clayden, N. J.; Williams, R. J. P. *J. Magn. Reson.* **1982**, *49*, 383.
(65) Williamson, M. P.; Asakura, T. *J. Magn. Reson.* **1991**, *94*, 557.
(66) Asakura, T.; Nakamura, E.; Asakawa, H.; Demura, M. *J. Magn. Reson.* **1991**, *93*, 355.

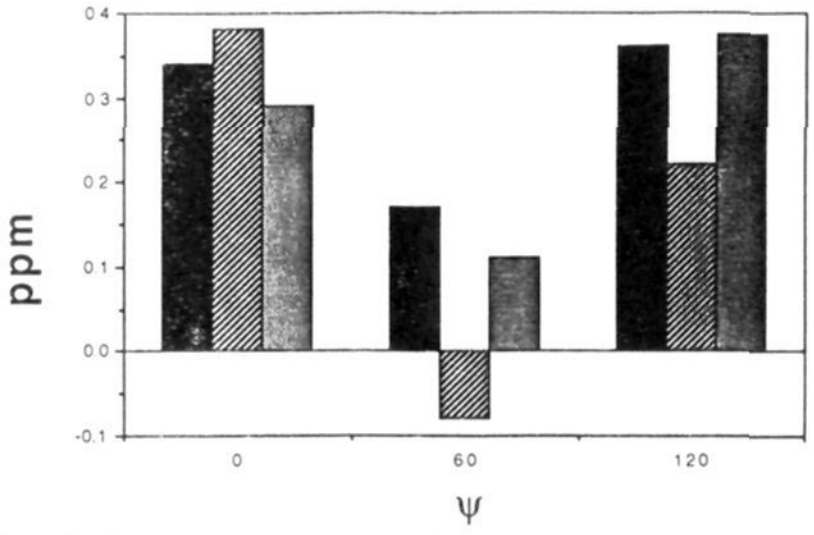


Figure 9. Difference in the absolute chemical shielding between the *pro-R*- and the *pro-S*-proton in glycine amide, computed at the GIAO-SCF/6-311G**//MP2/6-31G* level (first bar), GIAO-MP2/6-311G**//MP2/6-31G* level (second bar), and GIAO-SCF/6-311G**//RHF/6-31G* level of theory (third bar) for $\psi = 0°$, 60°, and 120°. (The first level corresponds to the left-most bar in each set.)

/RHF/6-31G*, GIAO-SCF/6-311G**//MP2/6-31G*, and GIAO-MP2/6-311G**//MP2/6-31G* levels of theory. Hence, to obtain a chemical shift surface which has almost MP2 quality, only the shielding of one conformation, C_{ref}, has to be computed at the GIAO-MP2 level. The shielding of any other conformation may be obtained by adding the difference between the GIAO-SCF shielding of C_{ref} and the conformation of interest to the MP2 result of C_{ref}.

Both **1** and **2** are suitable model systems for peptides. They are able to predict the change in the chemical shifts of C_α with respect to ψ correctly. The experimentally found difference between the chemical shielding that is found for the α-helix and the β-pleated sheet is reproduced. However, since the dependency on ϕ is neglected, this may be due to a furtuitous cancellation of the contribution of ϕ. The shift of the diastereotopic protons at C_α depends strongly on the relative orientation of the carboxy or carbamide moiety. A coplanar orientation of $O_{C=O}$ and a hydrogen at C_α has a deshielding effect on that proton, whereas if N_{amide} comes close to one of the hydrogens, the latter becomes more shielded. We suspect that the amide proton could be responsible for this effect. Studies on larger model systems are carried out to confirm our assumptions.

In this study we have only investigated a relatively small model system, which cannot mimic all properties of a peptide chain. However, even on the basis of our small system, we are able to predict the changes in the chemical shifts. Furthermore, we showed that the change in the chemical shielding of carbon, nitrogen, and oxygen with respect to the dihedral angle ψ is almost the same at the GIAO-SCF and GIAO-MP2 levels. Therefore we are convinced that the calculation of the NMR chemical shielding will develop into an important tool for the determination of the tertiary structures of peptides and proteins and supplement the existing methods.

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