The Carbonyl ^{13}C Chemical Shift Tensors of Five Peptides Determined from ^{15}N Dipole-Coupled Chemical Shift Powder Patterns

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Abstract: The ^{13}C chemical shift tensors have been determined for the glycine carbonyl carbon in a homologous series of peptides of the general form *N*-acetyl[1-^{13}C]glycyl-X-amide, where X was [^{15}N]glycine, DL-[^{15}N]-tyrosine, L-[^{15}N]phenylalanine and DL-[^{15}N]alanine. The principal values and molecular orientations of the tensors were extracted from ^{15}N dipole-coupled ^{13}C powder spectra. The shift tensor of a powdered sample of [1-^{13}C]glycyl[^{15}N]glycine·HCl·H_2O was determined by the same method and was found to agree to within 1 ppm in principal values and 2° in orientation with the previous single crystal measurements of R. E. Stark et al. (*J. Magn. Reson.* **1983**, *55*, 266). The shift tensors of the five peptides were found to be significantly different in both principal values and molecular orientation. However, the isotropic chemical shifts of the end-protected peptides in D_2O were nearly identical. From these data it is concluded that lattice environment has a significant effect on the chemical shift tensors of peptide carbonyl carbons. An approach to approximating carbonyl ^{13}C chemical shift tensors of peptides in proteins with use of the isotropic chemical shift in the molecule of interest is proposed. In addition, the utility of the powder pattern technique for accurately determining the chemical shift tensors of peptide carbonyl carbons is demonstrated.

Orientation-dependent phenomena such chemical shift anisotropy and dipolar coupling, observable in the NMR spectra of molecules in the solid state, provide a wealth of information that can be interpreted in terms of molecular structure. In the case of biomolecules, solid-state NMR has proven useful in structural studies of DNA,[2] proteins,[3] viruses,[4] and lipid bilayers.[5] The NMR spectra of oriented molecules can be very sensitive to the orientation of the molecule with respect to the magnetic field. Small changes in molecular orientation often lead to readily detected changes in chemical shift or dipolar splitting. The interpretation of the NMR spectra of these systems in terms of molecular orientations is critically dependent on an accurate knowledge of the principal values and orientation of the chemical shift or dipolar tensors in the molecule of interest.

The ^{13}C chemical shift anisotropy of peptide carbonyl carbons has the potential to provide information about the relative orientations of peptide planes in oriented[4] or anisotropically rotating protein molecules.[3] The insensitivity of peptide bond geometries to side chain moities[6] suggests that there could be a canonical shift tensor for all peptide carbonyl ^{13}C in proteins. It has been suggested that the only previously determined peptide carbonyl ^{13}C shift tensor, that of glycylglycine hydrochloride monohydrate, may represent such a tensor.[7] To test this idea, we have synthesized a homologous series of four end-protected dipeptides, *N*-acetyl-[1-^{13}C]glycyl[^{15}N]-X-amide (see Table I), and have determined the ^{13}C chemical shift tensors of the glycine carbonyl carbon in these molecules.

One reason for the paucity of data on peptide chemical shift tensors is that the standard method for obtaining them requires a large (tens of milligrams) single crystal and knowledge of the crystal structure.[8] In the case of our end-protected dipeptides, attempts at crystal growth were unsuccessful. An alternative to the single-crystal method involves the use of powdered samples. The NMR spectrum of an isolated nucleus in a microcrystalline or amorphous powder contains features that can be directly related to the principal values of its chemical shift tensor,[7] but the orientation of the tensor cannot be determined from this sort of spectrum. However, when the nucleus is dipole-coupled to a second nearby nucleus, the dipolar tensor is manifested in the powder pattern as well. As first pointed out by Kaplan et al.[9] for a $^{13}C/^{15}N$ pair in acetonitrile, this dipole-coupled chemical shift powder pattern contains sufficient information to allow the determination of the relative orientation of the chemical shift and dipolar tensors. Because the dipolar tensor is an axially symmetric tensor whose unique axis is known to lie along the internuclear vector,[10] the orientation of the chemical shift tensor relative to this molecular axis can be directly determined from the dipole-coupled powder pattern. This principle has been applied to the determination of ^{13}C chemical shift tensors of several small molecules in argon matrices. It has also been used to determine chemical shift tensors from spectra obtained by several two-dimensional techniques in the form of dipolar-modulated spectra,[11] separated local field spectra,[12] two-dimensional powder patterns,[13] and dipolar/chemical

Table I. Peptide Molecules Used in This Study

	A-NHCH$_2$^{13}C(O)^{15}NCH(R)C(O)-B			
	A	R	B	abbreviation
1	CH_3CO	DL-CH_3(DL-Ala)	NH_2	AcGlyAlaNH_2
2	CH_3CO	DL-$CH_2C_6H_4OH$(DL-Tyr)	NH_2	AcGlyTyrNH_2
3	H	H(Gly)	OH	GlyGly·HCl
4	CH_3CO	H(Gly)	NH_2	AcGlyGlyNH_2
5	CH_3CO	L-$CH_2C_6H_5$(L-Phe)	NH_2	AcGlyPheNH_2

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(2) Shindo, H.; Fujiwara, T.; Akutsu, H.; Matsumoto, U.; Kyogoku, Y. *Biochemistry* **1985**, *24*, 887-895.

(3) Lewis, B. A.; Harbison, G. S.; Herzfeld, J.; Griffin, R. G. *Biochemistry* **1985**, *24*, 4671-4679.

(4) Cross, T. A.; Opella, S. J. *J. Mol. Biol.* **1985**, *182*, 367-381.

(5) Seelig, J. *Biochim. Biophys. Acta* **1978**, *515*, 105-140.

(6) Pauling, L.; Corey, R. B. *Fortschr. Chem. Org. Naturst.* **1954**, *11*, 180-239.

(7) Stark, R. E.; Jelinski, L. W.; Ruben, D. J.; Torchia, D. A.; Griffin, R. G. *J. Magn. Reson.* **1983**, *55*, 266-273.

(8) Mehring, M. *High Resolution NMR in Solids*; Springer-Verlag: New York, 1983; pp 19-25.

(9) Kaplan, S.; Pines, A.; Griffin, R. G.; Waugh, J. S. *Chem. Phys. Lett.* **1974**, *25*, 78-79.

(10) Abragam, A. *Principles of Nuclear Magnetism*; Oxford University Press: London, 1961; pp 97-106.

(11) Stoll, M. E.; Vega, A. J.; Vaughan, R. W. *J. Chem. Phys.* **1976**, *65*, 4093-4098.

0002-7863/87/1509-5956$01.50/0

shift magic angle spinning spectra.[14]

We have obtained ^{15}N-coupled ^{13}C powder patterns of the end-protected peptides and glycylglycine·HCl·H_2O, enriched in ^{13}C at the glycine carbonyl and ^{15}N at the amide nitrogen of the C-terminal residue. In contrast to previous suggestions,[7] we have found that the ^{13}C shift tensors of the five peptides studied differ considerably both in magnitude and molecular orientation. However, we also find that the isotropic chemical shifts of the four end-protected peptides in solution are nearly identical. These observations have led us to conclude that the differences observed in the shift tensors are the result of particular lattice-dependent interactions present in the solid form of each peptide.

Experimental Section

Peptide Synthesis. Four of the dipeptides used in this study were of the end-protected form, *N*-acetylglycyl-X-amide, where X was glycine (**1**), DL-alanine (**2**), L-phenylalanine (**3**), and DL-tyrosine (**4**). The fifth dipeptide was the HCl salt of unprotected glycylglycine (**5**) (see Table I). In all cases, the N-terminal glycine was enriched to 90% ^{13}C at the carbonyl carbon. The X residue in all cases was 99% enriched in ^{15}N at the amino nitrogen. The dipeptides were synthesized in solution, using carbonyldiimidazole as the coupling reagent. Because the reaction conditions varied depending on the X residue, their syntheses are described separately below.

***N*-Acetylglycine (6).** As an intermediate in the syntheses described below, *N*-acetylglycine was synthesized by acetylation of ^{13}C-carbonyl glycine (MSD Isotopes).[15]

***N*-acetylglycyl-DL-alaninamide (1).** To a 5-mL round-bottomed flask equipped with a small stir bar was added 0.164 g (0.0014 mol) of **6**, 0.226 g (0.0014 mol) of *N,N'*,1,1'-carbonyldiimidazole,[16] and 1.5 mL of dry chloroform. The reaction was allowed to stir slowly at room temperature. After 0.5 h, 0.214 g (0.0014 mol) of ethyl[^{15}N]-DL-alanate hydrochloride (prepared from ^{15}N-labeled alanine, MSD Isotopes[17]) was added and again the reaction mixture was allowed to stir slowly at room temperature. Twelve hours later, the solvent was removed by rotary evaporation, and 5 mL of 1 N HCl was added. The peptide ethyl ester, which is partially soluble in water and exists as an oil at the bottom of the flask, was then, sequentially, taken up with 3 × 5 mL of chloroform, washed with 3 mL of cold 5% bicarbonate solution, and dried with anhydrous sodium sulfate. The solvent was then removed in vacuo, yielding 0.186 g (0.0009 mol) (61%) of the solid white peptide ester. The amide was generated[18] and then recrystallized from methanol/water, yielding 0.108 g (0.0005 mol) (41% overall yield) of pure product **1** as determined by high-resolution ^1H, ^{13}C, and ^{15}N NMR spectroscopy.

***N*-Acetylglycyl-DL-tyrosinamide (2).** Ethyl-DL-[^{15}N]tyrosinate free base was prepared from 0.245 g (0.0010 mol) of ethyl-DL-tyrosinate hydrochloride (prepared from ^{15}N-labeled DL-tyrosine, MSD Isotopes[17]) on treatment with 1 equiv of sodium hydroxide in the form of 1 N NaOH followed by extraction of the free base into ethyl acetate. To a 5-mL round-bottomed flask was added a small stir bar, 0.018 g (0.0007 mol) of **6**, 0.096 g (0.0007 mol) of *N,N'*,1,1'-carbonyldiimidazole,[16] and 1 mL of dry chloroform. After 0.5 h of stirring under a nitrogen atmosphere, 0.143 g (0.0007 mol) of [^{15}N]-DL-tyrosine ethyl ester free base[17] was added, and the reaction mixture was allowed to stir slowly at room temperature. Twelve hours later, the solvent was removed by rotary evaporation, and 5 mL of 1 N hydrochloric acid was added. the peptide ethyl ester, a yellowish oil, was then taken up with 3 × 5 mL portions of ethyl acetate, which was subsequently combined, washed with 5 mL of cold 5% bicarbonate solution, and dried with anhydrous sodium sulfate, and the ethyl acetate was then removed in vacuo. Drying the amorphous glasslike solid thus obtained under high vacuum for 6 h yielded 0.79 g (0.00058 mol) (88%) of the peptide ester. The peptidamide was generated[18] and then loaded onto a Sephadex LH-20 column with 95% EtOH as the solvent (column size, 85 cm × 1.4 cm; flow rate, approximately 0.1 mL/min or one column volume/h). After workup, the peptide amide was crystallized from 100% EtOH, yielding 0.152 g (0.00054 mol) (80% overall yield) of product **2** in pure form as determined by high-resolution ^1H, ^{13}C, and ^{15}N NMR spectroscopy.

Glycylglycine Hydrochloride Monohydrate (3). To a 10-mL round-bottomed flask fitted with a stir bar was added 0.525 g (0.0025 mol) of carbobenzoxy[1-^{13}C]glycine (synthesized from ^{13}C-labeled glycine, MSD Isotopes[19]) and 0.405 g (0.0025 mol) *N,N'*,1,1'-carbonyldiimidazole,[15] followed by 2.5 mL of dry chloroform. The reaction was allowed to stir slowly at room temperature under a nitrogen atmosphere. After 0.5 h, 0.353 g (0.0025 mol) of [^{15}N]glycine ethyl ester hydrochloride (prepared from ^{15}N-labeled glycine, MSD Isotopes[17]) was added, and the reaction mixture was allowed to stir at a moderate speed at room temperature. After 12 h, the chloroform was removed via rotary evaporation, and then 10 mL of 1 N hydrochloric acid was added. The peptide ester, an oil at the bottom of the flask, was then taken up with 3 × 10 mL portions of ethyl acetate. The EtAc layers were then combined, washed with 5 mL of cold 5% bicarbonate solution, and dried over anhydrous sodium sulfate. The solvent was then removed by rotary evaporation, yielding 0.408 g (0.0014 mol) (55%) of peptide ester. The peptide ester was then saponified with NaOH[20] and methanol/water with paladium black catalyst.[21] The methanol was removed from the reaction mixture by rotary evaporation at 40 °C for 0.5 h, followed by the addition of 2 equiv of HCl (in the form of 1 N hydrochloric acid) to generate the hydrochloride. The water was removed via lyophilization, to yield 0.325 g (0.0017 mol) (68%) of peptide hydrochloride. The white amphorous solid thus obtained was recrystallized from double distilled water at room temperature, yielding 0.186 g (0.0010 mol) (40% overall yield) of pure product **3** as determined by high-resolution ^1H, ^{13}C, and ^{15}N NMR spectroscopy.

***N*-Acetylglycylglycinamide (4).** To a 10-mL round-bottomed flask was added 0.295 g (0.0025 mol) of **6** and 0.405 g (0.0025 mol) of *N,N'*,1,1'-carbonyldiimidazole, followed by 2.5 mL of dry chloroform. The reaction mixture was stirred slowly at room temperature under a nitrogen atmosphere. One-half hour later, 0.353 g (0.0025 mol) of [^{15}N]glycine ethyl ester hydrochloride (prepared from ^{15}N-labeled glycine, KOR Isotopes[17]) was added, and the stirring was continued at room temperature. Thirteen hours later, the peptide ethyl ester, which precipitated out of the chloroform, was collected on a Buchner funnel under vacuum and dried under a high vacuum to give 0.389 g (0.0019 mol) (76%) of product. The peptide ethyl ester was then converted to the amide in methanol.[18] The amide was found to precipitate out of the methanol as it was formed, and at the end of 3 days it was collected via vacuum filtration. The solid peptide amide was then recrystallized from methanol/water and dried under high vacuum, yielding 0.196 g (0.0011 mol) (44% overall yield) of pure product **4** as determined by high-resolution ^1H, ^{13}C, and ^{15}N NMR spectroscopy.

***N*-Acetylglycyl-L-phenylalaninamide (5).** Ethyl L-[^{15}N]phenylalanate free base (prepared from ^{15}N-labeled phenylalanine, MSD Isotopes[17]) was synthesized from 0.354 g of ethyl L-[^{15}N]phenylalanate hydrochloride[17] on treatment with 1 equiv of sodium hydroxide in the form of 1 N NaOH, followed by extraction of the free base into ethyl acetate. To a 5-mL round-bottomed flask, equipped with a stir bar, was added 0.130 g (0.0011 mol) of **6** and 0.78 g (0.0011 mol) of *N,N'*,1,1'-carbonyldiimidazole,[15] followed by 1.5 mL of dry chloroform. The reaction was kept under a nitrogen atmosphere and allowed to stir slowly at room temperature. One-half hour later, 0.211 g (0.0011 mol) of [^{15}N]-L-phenylalanine ethyl ester free base was added, and the reaction mixture was maintained at ambient temperature with slow stirring. Sixteen hours later, the chloroform was removed via rotary evaporation, and then 10 mL of 1 N HCl was added. The dipeptide ethyl ester, present as an oil in the bottom of the flask, was then taken up with 3 × 10 mL portions of ethyl acetate. The EtAc layers were then combined and washed once with 5% cold bicarbonate solution, and then dried over sodium sulfate, and the solvent was removed in vacuo. The product, as an oil, was dried under a high vacuum for 6 h, to give 0.263 g (0.0009 mol) (82%) of peptide ethyl ester. The amide was then generated[18] and purified on a Sephadex LH-20 column with 95% EtOH as a solvent. After workup, the peptide amide (still an oil) was crystallized from 100% EtOH, giving 0.175 g (0.0007 mol) (60% overall yield) of pure product **5** as determined by high-resolution ^1H, ^{13}C, and ^{15}N NMR spectroscopy and reverse-phase HPLC on a C-18 column eluted with a gradient of acetonitrile/water.

NMR Experiments. Powder Spectra. The ^{13}C powder pattern spectra were obtained at 50.3 MHz with a Bruker CXP-200 Console and 4.7-T magnet with a probe equipped with a triply tuned solenoid coil[22] (6 × 20 mm). The spectra were collected with use of cross polarization with a mixing time of 5 ms and high-power CW ^1H decoupling with $(\gamma H_1)/2\pi$ = 109 kHz. A spin–echo pulse sequence was used to minimize distortions

(12) Hester, R. K.; Ackerman, J. L.; Neff, B. L.; Waugh, J. S. *Phys. Rev.* **1976**, *36*, 1081–1083.
(13) Linder, M.; Hohener, A.; Ernst, R. R. *J. Chem. Phys.* **1980**, *73*, 4959–4970.
(14) Munowitz, A.; Aue, W. P.; Griffin, R. G. *J. Chem. Phys.* **1982**, *77*, 1686–1689.
(15) Herbst, R. D.; Shemin, D. *Org. Synth.* **1939**, *19*, 4–5.
(16) Paul, R.; Anderson, G. W. *J. Am. Chem. Soc.* **1960**, *82*, 4596–4600.
(17) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 433–454.
(18) Chambers, R. W.; Carpenter, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 1522–1526.
(19) Greenstein, J. P.; Winitz, M. *Chemistry of* the *Amino Acids*; J. Wiley and Sons: New York, 1961; Vol. 2, p 891.
(20) Reference 19, Vol. 2, pp 1158–1159.
(21) Reference 19, Vol. 2; pp 1232–1235.
(22) Kan, S.; Fan, M.; Courtieu, J. *Rev. Sci. Instrum.* **1980**, *51*, 887–890.

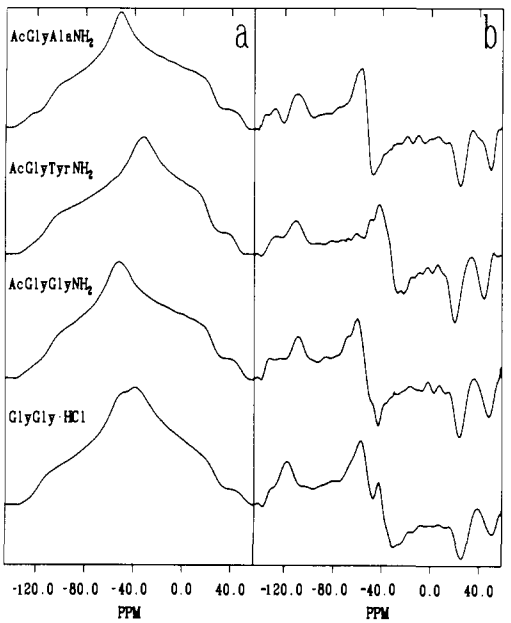

Figure 1. The ^{15}N dipole-coupled powder spectra of the ^{13}C-labeled carbonyl carbon of glycine in peptides **1–4**. The absorption spectra are shown in part a and the first derivative with respect to frequency in part b. Chemical shifts are relative to external liquid benzene (low ppm is downfield).

caused by pulse "breakthrough". The time between cross-polarization period and the ^{13}C π pulse was 20 μs. The pulses and receiver were phase cycled to remove artifacts caused by phase inaccuracies in the ^{13}C π pulse and residual, nonechoing FID. Recycle delays ranged from 8 to 30 s, and the number of acquisitions from 1500 to 3000, depending on the dipeptide, to give spectra with comparable signal-to-noise ratios. The total sweep width was 100 kHz, and 512 data points in each of the two channels in quadrature were collected. The sample sizes were approximately 150 mg. External hexamethyl benzene was used as a chemical shift standard, and chemical shifts were referenced to liquid benzene assuming the methyl peaks of hexamethylbenzene have a chemical shift of 111.5 ppm.

Solution Spectra. The high-resolution ^{13}C spectra were collected at 90.8 MHz on a standard Nicolet NT-360 instrument. The spectra were collected with a simple $\pi/2$ pulse sequence, using ^{1}H noise decoupling. The dipeptides were dissolved in D_2O at concentrations of ca. 50 mM with 0.1% dioxane as an internal chemical shift reference. ^{15}N was not decoupled.

MAS Spectra. The ^{13}C magic angle sample spinning (MAS) spectra were collected at 75.5 MHz on a GE 300 instrument equipped with a magic angle spinning probe with an Andrew-Beams type spinner.[23] Data were collected with use of cross polarization with a mixing time of 5 ms and high power ^{1}H decoupling with $(\gamma H_1/2\pi) = 45$ kHz. For measurement of isotropic chemical shifts, spinning speeds of 3 kHz or greater were used. For slow-spinning experiments, the spinning speed was $\sim$1 kHz, giving rise to at least five sidebands on either side of the center band.

Data Analysis. Analysis of the solid-state NMR spectra was done on a VAX 11/750, using a command driven NMR data analysis software package (FTNMR) as well as original FORTRAN programs whose algorithms are described below. In order to produce derivative spectra (see below) with sufficient signal-to-noise ratios for use in least-squares fitting it was necessary to apodize the time-domain spectra with a Kaiser window digital filter ($\alpha = 4$, $N = 2.5$ ms).[24] This apodization function effectively removed the noise at long times in the time domain spectrum (to which derivative spectra are very sensitive) without significantly broadening the frequency domain spectrum. No other line broadening was used.

Results

Powder Spectra. The ^{13}C NMR powder spectra of peptides **1–4** at 50.3 MHz are presented in Figure 1a. The shapes of these powder spectra reflect both carbon chemical shift and ^{15}N–^{13}C dipolar interactions. The first derivatives with respect to frequency of the spectra in Figure 1a are shown in Figure 1b. These derivative spectra were determined by using a point-by-point fit of the absorption spectra to a fifth-order polynomial and calculation of the derivative from the polynomial coefficients. Although NMR powder spectra obtained by continuous wave methods are often displayed in the first-derivative form, this practice is less common for spectra obtained by pulsed Fourier transform methods. However, in cases where information is contained primarily in the frequency of the edges, as in the present study, the first and higher order derivatives of the powder spectra are very useful. The spectra in Figure 1b, although lower in signal to noise, clearly show peaks corresponding to the downfield and upfield edges of the carbon spectrum, split into doublets by the ^{15}N adjacent to the labeled carbon.

A microcrystalline powder of a molecule containing an isolated spin $^1/_2$ nucleus has an NMR spectrum that in general consists of a broad signal with a single maximum and two inflection points. The frequencies of these features correspond to the three principal values of the chemical shift tensor: σ_{11}, σ_{22} and σ_{33} corresponding to the downfield inflection point, the maximum, and the upfield inflection point, respectively.[25] The powder spectrum of a spin $^1/_2$ nucleus dipole coupled to a second spin $^1/_2$ nucleus contains, in general, four inflection points and two maxima (although one maximum may appear as an inflection point). These features correspond to the dipolar doublets arising from molecular orientations for which the magnetic field vector lies parallel to the principal axes.[26] Thus, the two upfield inflection points evident in the spectra in Figure 1a correspond to the dipolar doublet pairs for molecular orientations which place the magnetic field along σ_{33}. The frequency of these inflection points is readily seen in the derivative spectra, shown in Figure 1b. However, due to intensity from dipolar doublets of other molecular orientations, the frequencies do not directly correspond to the chemical shift and dipolar splitting. These parameters must be extracted by iterative fitting of the spectra.

As mentioned above, several authors have shown that, in principle, dipole-coupled chemical shift powder patterns contain enough information to allow the determination of the orientation of the dipolar vector (in this case, the C–N amide bond) in the principal axis system of the chemical shift tensor.[9,11-13,26] This information is sufficient to place the chemical shift tensor in the molecular coordinate system in cases where molecular symmetry suggests the orientation of one of the three principal components. In a peptide, the amide bond has an approximate plane of symmetry containing the carbonyl oxygen and carbon and amide nitrogen atoms. This implies that one of the principal components of the shift tensor is perpendicular to the peptide plane. Previous studies on carbonyl compounds,[27] together with analysis of the powder patterns in the present study, indicate that σ_{33} is approximately perpendicular to the peptide plane and that σ_{11} and σ_{22} lie in the peptide plane. Determination of the orientation of the chemical shift tensor in the peptide coordinate system under these conditions reduces to the measurement of the angle between σ_{11} and the C–N bond.

We have used an iterative method-of-steepest-descent[28] least-squares fitting procedure to determine the principal values and orientation of the chemical shift tensor of the carbonyl carbon of glycine in peptides **1–4**. This procedure is discussed in detail elsewhere[29] and will be described only briefly here. Our method of simulation of chemical shift powder patterns, which is based

(23) Bartuska, V. J.; Maciel, G. E. *J. Magn. Reson.* **1981**, *42*, 312–317.
(24) Hamming, R. W. *Digital Filters*; Prentice-Hall: Englewood Cliffs, NJ, 1983; pp 171–176.
(25) Haeberlen, U. *Advances in Magnetic Resonance, Suppl. 1: High Resolution NMR in Solids: Selective Averaging*; Academic: New York, 1976; pp 24–30.
(26) Zilm, K. W.; Grant, D. M. *J. Am. Chem. Soc.* **1981**, *103*, 2913–2922.
(27) (a) Pines, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys.* **1972**, *56*, 1776. (b) Pines, A.; Gibby, M. G.; Waugh, J. S. *Chem. Phys. Lett.* **1972**, *15*, 373. (c) Kempf, J.; Speiss, H. W.; Haeberlen, U.; Zimmermann, H. *Chem. Phys.* **1972**, *17*, 39. (d) Pausak, S.; Pines, A.; Waugh, J. S. *J. Chem. Phys.* **1973**, *59*, 591.
(28) Bevington, P. R. *Data Reduction and Error Analysis for the Physical Sciences*; McGraw-Hill: New York, 1969; pp 219–222.
(29) Oas, T. G.; Drobny, G. P., Dahlquist, F. W., in preparation.

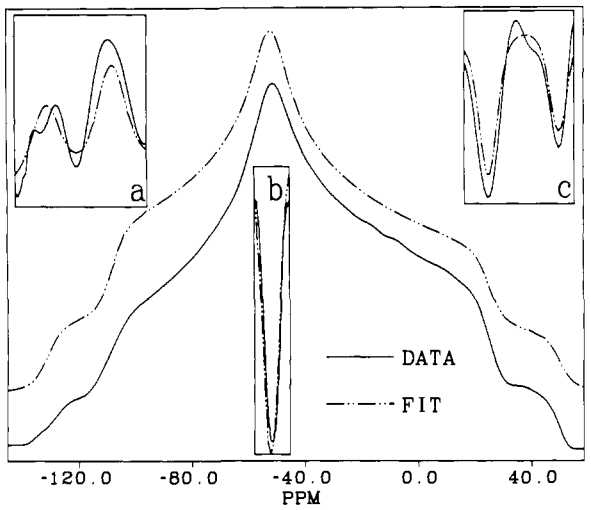

Figure 2. A comparison of the actual and simulated powder spectra for $AcGlyAlaNH_2$. Insets a–c show regions used to fit σ_{33}, σ_{22}, and σ_{11}, respectively. Region c was used to fit the dipolar coupling constant, while regions a and b were used to determine the orientation of the chemical shift tensor in the peptide plane. The Lorentzian and Gaussian line broadenings were fit independently for each region. Regions a and c were fit in the first derivative form and region b was fit in the second derivative form. The integral of the simulation was normalized to that of the data for each region and is displayed as such in the figure. Chemical shifts are relative to external liquid benzene (low ppm is downfield).

in part on methods of other workers,[30] involves the calculation of the following form of the powder spectrum

$$s(\omega) = \sum_{\phi=0}^{\pi} \sum_{\theta=0}^{\pi} g(\omega_0(\theta,\phi) - \omega) \quad (1)$$

where $g(\omega_0(\theta,\phi) - \omega)$ is the Voigt profile line shape function.[31] $\omega_0(\theta,\phi)$ is given by the equation

$$\omega_0 = (\sin^2\theta\cos^2\phi)\sigma_{11} + (\sin^2\theta\sin^2\phi)\sigma_{22} + (\cos^2\theta)\sigma_{33} \pm \Delta\omega/2 \quad (2)$$

where σ_{11}, σ_{22}, and σ_{33} are the principal components of the chemical shift tensor. $\Delta\omega$ is given by the equation

$$\Delta\omega = D[1 - 3\{\sin\beta\sin\theta\cos(\phi - \alpha) + \cos\beta\cos\theta\}^2] \quad (3)$$

where β and α are the polar and azmithul angles, respectively, of the C–N internuclear vector in the axis system of the chemical shift tensor, and D is the dipolar coupling constant ($\gamma_C\gamma_N\hbar/r_{CN}^3$). In our simulations, θ and ϕ are stepped in equal area increments of the unit sphere (1 deg step sizes in θ, sin θ deg steps in ϕ), producing the frequency domain powder spectrum described by eq 1.

Figure 2 shows the data and best-fit simulation of $AcGlyAlaNH_2$ (**1**). Variations in the relative intensities of data and fit are apparent across the powder pattern. The insets in Figure 2 show the actual regions that were iteratively fit with a gradient least-squares minimization routine.[28] First, the region shown in Figure 2c, in the first derivative form, was fit by varying σ_{33}, the dipolar coupling constant, and the line shape[32] due to each orientation. The region shown in Figure 2a, also in the first derivative form, was then fit varying σ_{11}, the angle between the C–N bond, and the line shape. Finally the region shown in Figure 2b, in the second derivative form, was fit varying σ_{22}, the angle between the C–N bond, and the line shape. In all cases the best-fit orientation angle determined from fitting regions a and b in Figure 2 agreed to within 2°. This approach was applied to the powder spectra of peptides **1–4**, and the resultant best-fit parameter values are summarized in Table II.

Table II. Glycine ^{13}C Carbonyl Chemical Shift Tensors of Five Peptides[a]

molecule	principal values (ppm) σ_{11}	σ_{22}	σ_{33}	angle between σ_{11} and the ^{13}C–^{15}N Bond[b] α (deg)
$AcGlyAlaNH_2$	−113.6	−56.4	38.5	−36.6
$AcGlyTyrNH_2$	−114.0	−37.0	33.2	−40.7
GlyGly·HCl	−115.3	−48.7	39.4	−46.6
$AcGlyGlyNH_2$	−114.5	−55.7	37.3	−34.5
$AcGlyPheNH_2$-1[c]	−117.0	−45.7	34.9	
$AcGlyPheNH_2$-2	−117.0	−39.3	35.4	

[a] Relative to external liquid benzene (low ppm values are downfield). Uncertainties are ±1.5 ppm. [b] σ_{11} and σ_{22} are assumed to lie in the peptide plane (see text). Uncertainties are ±2°. [c] Determined from sideband analysis of the MAS spectrum.[35] The presence of ^{15}N dipolar coupling probably causes an overestimation of σ_{33}–σ_{11}. In addition to this systematic error, uncertainties are ±2 ppm. Tensors 1 and 2 are for the downfield (1) and upfield (2) major peaks in Figure 3.

The dipolar splittings at the regions of the powder pattern corresponding to σ_{11}, σ_{22}, and σ_{33} are not linearly independent because the dipolar coupling of three orthogonal orientations must sum to zero.[33] This fact prevents the unique determination of the dipolar coupling constant (D) and the shift tensor orientation solely from fitting these regions. In principle, other regions are independently sensitive to coupling constant and shift tensor orientation. However, in the powder patterns of peptides **1–4** at 50.3 MHz these other regions contribute to such a small fraction of the total intensity that it is impossible to fit to them. In simulations of the spectra in Figure 1, values of β less than 90° can be compensated for by increasing D without significantly altering the distinguishable features of the spectrum. Nevertheless, on the basis of C–N amide bond lengths of known peptide structures,[34] a lower limit of 1150 Hz (a bond length of 1.39 Å) can be assumed for D.[10] This restriction confines β to between 80° and 90° based on the splittings observed at the σ_{33} edges in the powder pattern. Fits of the upfield edges (region c, Figure 2) of peptides **1–4** with β fixed at 90° have yielded dipolar coupling constants ranging from 1225 to 1260 Hz, which corresponds to bond lengths ranging from 1.34 to 1.35 Å, well within the range of observed amide bond lengths.[34] These results are consistent with our assumption that σ_{33} lies perpendicular to the peptide plane, although any chemical shift tensor orientation corresponding to rotation of the tensor about the C–N bond would result in identical dipole-coupled powder spectra.

As is qualitatively evident from comparing the spectra in Figure 1, the data shown in Table II indicate significant differences in the principal values of the chemical shift tensors of peptides **1–4**. This is particularly true for σ_{22}, which ranges from −56 ppm for $AcGlyAlaNH_2$ to −37 ppm for $AcGlyTyrNH_2$. As will be shown below, these differences cannot be accounted for on the basis of the chemical nature of the R group. The data listed in Table II for the angle (α) between the C–N bond and σ_{11} also show that there are significant differences in the orientation of the chemical shift tensors for peptides **1–4**. For the four peptides studied, there is a range of 12.5° in the orientation of σ_{11} and σ_{22} in the peptide plane. The data given in Table II for GlyGly·HCl agree very well (within 1 ppm in principal values and 1° in orientation) with the results of a previous single-crystal study on the same molecule.[7]

Magic Angle Spinning Spectra. The magic angle spinning (MAS) spectra of peptides **1–5** were obtained in order to confirm the powder pattern results. The MAS spectrum of $AcGlyPheNH_2$, shown in Figure 3, contains at least three resolvable peaks rather

(30) (a) M. Munowitz, personal communication. (b) Reference 10.
(31) Hui, A. K.; Armstrong, B. H.; Wray, A. A. *Quantum Spectrosc. Radiat. Transfer* **1978**, *19*, 509–516.
(32) The Voight profile (convolution of Gaussian and Lorentzian line shapes) is chosen to approximate the actual line shapes which are presumed to contain contributions from T_2-related processes (Lorentzian) and sample inhomogenities (Gaussian). This line shape function can be computed rapidly with a numerical method that approximates the complex error function (see ref 31).
(33) The dipolar coupling tensor is Hermitian (ref 10) and can be transformed via a unitary transformation to the principal axis system of the chemical shift tensor. Under these conditions, the dipolar couplings of orientations along these axes are invariant and because the dipolar tensor is traceless must sum to zero.
(34) Benedetti, E. *Chem. Biochem. Amino Acids, Pept., Proteins* **1982**, *6*, 105–184.

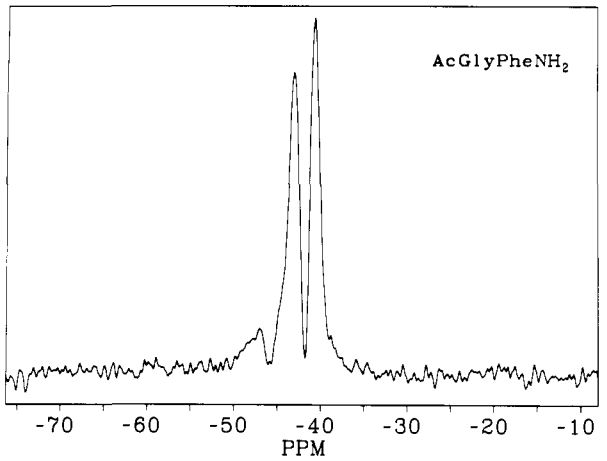

Figure 3. The MAS spectrum of $AcGlyPheNH_2$ showing three distinguishable isotropic chemical shifts present in the sample. This figure demonstrates the existence of three different chemical shift tensors for the same molecule (see text). Chemical shifts are relative to external liquid benzene (low ppm is downfield).

Table III. Comparison of Isotropic Chemical Shifts of Five Peptides As Determined from Powder Patterns, MAS Spectra, and Solution NMR Spectra

	isotropic chemical shift (ppm)		
peptide	powder pattern[a]	MAS[b]	solution[c]
$AcGlyAlaNH_2$	-43.8	-43.7	-43.3
$AcGlyTyrNH_2$	-39.3	-38.7	-43.3
GlyGly·HCl	-41.5	-40.7	-39.4
$AcGlyGlyNH_2$	-44.3	-44.1	-44.2
$AcGlyPheNH_2$-1		-42.6	-43.3
$AcGlyPheNH_2$-2		-40.5	-43.3

[a] Chemical shifts are calculated from data in Table 2 with $\sigma_{iso} = 1/3Tr(\sigma)$. Uncertainties are ±1.5 ppm. [b] Relative to external liquid benzene. Uncertainties are ±0.5 ppm. [c] Determined in D_2O with *p*-dioxane as an internal standard. Chemical shift of *p*-dioxane relative to liquid benzene assumed to be -61.5 ppm. Uncertainties are ±0.1 ppm.

than a single peak, which was found in the spectra of peptides **1–4**. Careful checking of the peptide's purity by HPLC and by solution ^1H, ^{15}N, and ^{13}C NMR spectroscopy indicated the presence of only one chemical species. These results indicate that three different shift tensors for the labeled carbonyl carbon must be present in the sample. This might be the case if there were three different crystal forms present in the powder (see Discussion). The chemical shifts of the two major components (of approximately equal intensity) of $AcGlyPheNH_2$ are listed in Table III, along with the chemical shifts of the single peaks in the MAS spectra of peptides **1–4**.

As expected from the powder pattern results, the MAS chemical shifts vary significantly from -38.7 ppm for $AcGlyTyrNH_2$ to -44.1 ppm for $AcGlyGlyNH_2$ (relative to liquid benzene, low ppm values downfield), a range of 5.4 ppm. The isotropic chemical shifts calculated from the averages of the principal values for peptides **1–4** obtained by powder pattern fitting agree with the MAS results, within experimental error (see Table III).

In addition to fast-spinning MAS, slow-spinning spectra containing at least five sidebands on either side of the center band were obtained for peptides **1–5**. These spectra were subjected to analysis by the method of Herzfeld and Berger[35] to obtain estimates of the shift tensor principal values for all five peptides. Application of this method is not entirely valid in this case because it was not designed to be applied to samples where heteronuclear dipolar coupling is left undecoupled. However, since the maximum dipolar coupling constant in this case is approximately 10% of $\sigma_{33}-\sigma_{11}$, the error should be small enough to allow comparison of the principal values obtained by powder pattern fitting and

(35) Herzfeld, J.; Berger, A. E. *J. Chem. Phys.* **1980**, *73*, 6021–6030.

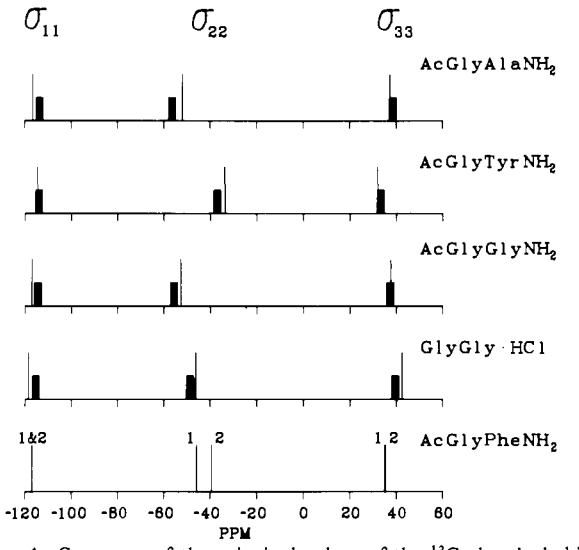

Figure 4. Summary of the principal values of the ^{13}C chemical shift tensors of peptides **1–5** as determined by (1) iterative fitting of the powder patterns (thick bars) and by (2) analysis of sideband intensities in slow-spinning MAS spectra (thin bars). The uncertainty in the fitting results is indicated by the width of the bars (±1.5 ppm). The uncertainty in the MAS results is ca.±2 ppm. Chemical shifts are given relative to liquid benzene (low ppm is downfield).

sideband analysis. Figure 4 shows the values obtained by the two techniques. In most cases the principal values obtained by the two methods agree within experimental error, although the sideband analysis seems to underestimate σ_{11} and overestimate σ_{22}. The principal values σ_{11} and σ_{33} are identical, within experimental error, for the two major crystal forms of $AcGlyPheNH_2$, as determined by sideband analysis. This result is consistent with the relative invariance of these values among other carbonyl tensors and indicates that the measured difference of 6.4 ppm in σ_{22} for the two crystal forms is accurate, even if the actual values are not (see Table II).

Solution Spectra. Table III gives the chemical shifts of peptides **1–5** in D_2O solution. The solution chemical shifts of peptides **3–5** are identical within experimental error. The solution chemical shift of $AcGlyGlyNH_2$ differs from these by less than 1 ppm. The solution chemical shift of GlyGly·HCl is significantly different from the end-protected dipeptides, presumably reflecting the presence of the charged amino group at low pH.

The solid and solution isotropic chemical shifts shown in Table III are within experimental error for $AcGlyAlaNH_2$ and $AcGlyGlyNH_2$. However, the isotropic shifts for the solution and solid forms of the other peptides are significantly different. These differences must reflect a significant change in the carbonyl shift tensors of peptides **2**, **3**, and **5** when they are dissolved in D_2O.

Discussion

The powder pattern fitting method used in determining the chemical shift tensors in this study has proven very sensitive to both the shift tensor principal values and the orientation of the ^{13}C–^{15}N internuclear vector in the (σ_{11}, σ_{22}) plane. In these respects, the method is probably as accurate as the single-crystal method.[8] The excellent agreement between the shift tensor of GlyGly·HCl determined by the single-crystal method (σ_{11} = -115.6, σ_{22} = -48.6, σ_{33} = 40.6 ± 1.5 ppm, α = -48.6°)[7] and our results indicates that the method used here has produced reliable tensors for all of the molecules studied. This conclusion is reinforced by the agreement in isotropic chemical shifts as calculated from the shift tensor principal values and those determined from MAS spectra (see Table III). The reliability of the method used to determine chemical shift tensors in this study is important because as will be shown it is one of the few approaches available for determining chemical shift tensors (both magnitude and orientation) in samples that cannot be obtained in a single-crystal form. Other approaches to determining chemical shift tensors in powdered or amorphous samples include chemical shift–dipolar

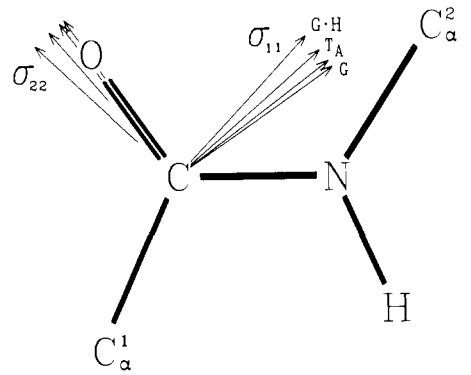

Figure 5. The orientations of σ_{11} and σ_{22} in the peptide plane, assuming σ_{33} is perpendicular to the plane, for peptides **1–4**. The abbreviations refer to the following: A = $AcGlyAlaNH_2$ (**1**), T = $AcGlyTyrNH_2$ (**2**), G = $AcGlyGlyNH_2$ (**3**), G·H = GlyGly·HCl (**4**).

MAS spectroscopy[14] and techniques that utilize dipolar-modulation of chemical shift powder patterns.[11-13] The method used here has an advantage over these techniques in its instrumental simplicity.

The principle values of the chemical shift tensors of peptides **1–5** as determined by powder pattern fitting and spinning sideband analysis are displayed in Figure 4. There is no significant difference in σ_{11} among the five peptides. There is also no significant difference in σ_{33} for $AcGlyAlaNH_2$, $AcGlyGlyNH_2$, and GlyGly·HCl. However, the σ_{22} values show a large variation for the five dipeptides studied. These results are consistent with the observations of previous workers, which indicate that most of the differences in carbonyl ^{13}C chemical shift tensors are due to σ_{22}.[27] In addition, these results indicate that the range of isotropic ^{13}C chemical shifts of peptide carbonyls in proteins is produced mostly by variations in σ_{22}.

Figure 5 shows the orientation of σ_{11} and σ_{22} in the peptide plane for peptides **1–4**. For $AcGlyGlyNH_2$, σ_{22} is parallel to the carbonyl bond. The orientation of the chemical shift tensor of $AcGlyAlaNH_2$ is very similar to that of $AcGlyGlyNH_2$. However, the $AcGlyTyrNH_2$ and GlyGly·HCl shift tensors have significantly different orientations with their σ_{22} axes rotated counterclockwise from the carbonyl bond by angles of 6° and 12°, respectively. There does not seem to be a direct correlation between the value of σ_{22} and the orientation of the shift tensor in these peptides since GlyGly·HCl has the most extreme tensor orientation while having an intermediate σ_{22} value.

Although the solution chemical shifts of peptides **2–5** are nearly identical, their isotropic shifts in the solid state differ by 5 ppm. These results, combined with the MAS spectrum of $AcGlyPheNH_2$ (Figure 3), which clearly shows multiple isotropic chemical shifts for the same molecule, lead us to conclude that the chemical shift tensors of the peptide molecules studied are significantly influenced by the lattice environment in which they are found. The chemical shift tensors of $AcGlyAlaNH_2$, $AcGlyTyrNH_2$, and $AcGlyPheNH_2$ in solution are probably identical, based upon their solution chemical shifts. This result implies that the variation in the principal values of these dipeptides in the solid form is due to intermolecular interactions such as hydrogen bonding. This phenomenon has been well documented in the ^{13}C chemical shift tensors of carbonyl and carboxyl groups of other compounds[36] in polypeptides containing ^{13}C carbonyl labeled glycine.[40] Molecular orbital calculations of the paramagnetic contribution to σ for carbonyl carbons have led Kempf et al.[37] to conclude that the variations observed in σ_{22} for different molecules are due primarily to variations in the excitation energy and MO coefficient of the $n \rightarrow \pi^*$ carbonyl electronic transition. Takegoshi et al.[36] have concluded that a large part of the variation in the $n \rightarrow \pi^*$ transition in carbonyl compounds may be due to different strengths of hydrogen bonds to the carbonyl oxygen. It is plausible that the variations in σ_{22} observed for the peptides in this study are due to different degrees of hydrogen bonding to the carbonyl oxygen, depending on the crystal structure of each peptide. In solution, the hydrogen bonding to D_2O would be essentially the same for each peptide, thus giving rise to identical shift tensors. Other possible lattice-dependent effects would include intermolecular ring current shifts in the case of the aromatic peptides and deviations in the planarity of the peptide group caused by crystal-induced packing forces.

Regardless of the precise nature of the lattice-dependent phenomena that cause the shift tensors of peptides **1–5** to differ, it is clear from the evidence presented here that the crystal lattice significantly alters the carbonyl ^{13}C shift tensors of peptides in the solid state. This conclusion calls into question the use of chemical shift tensors of model compounds in studies that deduce the orientation of molecular and motional axes from ^{13}C carbonyl chemical shift measurements.[3] For example, assume that a ^{13}C carbonyl labeled peptide group in a protein were oriented with its C=O bond parallel to the magnetic field. Assume also that the chemical shift tensor of this peptide group were the same as that of $AcGlyTyrNH_2$. The observed chemical shift of this peptide would be 37.9 ppm. If the orientation of the peptide were to be calculated from this chemical shift by assuming that the chemical shift tensor of GlyGly·HCl applied, the predicted orientation of the C=O bond would be in error by 22.5°.[38]

This error can be reduced if the isotropic chemical shift of the carbonyl ^{13}C can be determined in the molecule of interest. In the case of soluble proteins, the solution chemical shift of the particular peptide carbonyl would probably accurately reflect the average of the three principle values of its shift tensor. In solid proteins, the average could be determined from MAS spectra. This average (isotropic) chemical shift can be combined with assumed values of σ_{11} and σ_{33} to obtain an approximate value of σ_{22}. The average values of σ_{11} and σ_{33} for peptides **1–4** are $\langle\sigma_{11}\rangle = -114$ ppm (standard deviation = 0.7 ppm) and $\langle\sigma_{33}\rangle = 37$ ppm (standard deviation = 2.7 ppm), relative to liquid benzene. These values lead to the following approximation of σ_{22} for peptide carbonyl carbons

$$\sigma_{22} \simeq 3\sigma_{iso} + 77 \text{ ppm} \quad (4)$$

where σ_{iso} is the measured isotropic chemical shift in ppm relative to liquid benzene, with negative ppm values downfield. With use of this approximation and the isotropic chemical shift as determined by MAS, the predicted σ_{22} values for peptides **1–4** would be −54.1, −39.1, −45.1, and −55.3 ppm, respectively. Comparison of these values with those listed in Table I reveals agreement to within 3.6 ppm, which represents the minimum uncertainty in determining σ_{22} by this approach. The uncertainty for all peptides is likely to be larger, although it is probably much smaller then the range of σ_{22} seen in this study, which is almost 20 ppm. The example given above can be repeated by using principle values $\langle\sigma_{11}\rangle$, $\langle\sigma_{33}\rangle$, and σ_{22} calculated from σ_{iso} for $AcGlyTyrNH_2$ (from MAS) instead of the principal values of GlyGly·HCl. Assuming that the shift tensor has the same orientation as that of GlyGly·HCl, the predicted orientation of the C=O bond would be in error by 11.3°, half of the original error. This remaining error is mostly due to the residual 2.1-ppm discrepancy between the calculated and actual σ_{22} values.

Another application of eq 4 is in the interpretation of high-resolution NMR spectra of peptide carbonyl carbons in proteins. The chemical shift range of the peptide carbonyl signal in soluble

(36) Takegoshi, K.; Naito, A.; McDowell, C. A. *J. Magn. Reson.* **1985**, *65*, 34–42.
(37) Kempf, J.; Speiss, H. W.; Zimmermann, H. *Chem. Phys.* **1974**, 269–276.
(38) There is an infinite set of orientations that have a chemical shift of −37.9 ppm, assuming the chemical shift tensor of GlyGly·HCl. We have chosen a specific orientation by assuming that a second tensorial interaction can be observed. The $^{13}C-^{15}N$ dipolar coupling of a doubly labeled peptide oriented with its carbonyl bond parallel to the magnetic field would be −345 Hz, assuming standard peptide geometries (ref 10 and 34). The orientation calculated in this example corresponds to the intersection of the −37.9 ppm chemical shift and −345 Hz dipolar coupling isochromates in the principal axis system of the GlyGly·HCl chemical shift tensor.
(39) Kainosho, M.; Takashi, T. *Biochemistry* **1982**, *21*, 6273–6279.
(40) Ando, S.; Yamanobo, T.; Ando, I.; Shoji, A.; Ozaki, T.; Tabeta, R.; Saito, H. *J. Am. Chem. Soc.* **1985**, *107*, 7648–7652.

proteins is at least 10 ppm. It is likely that most of this range is due to variations in σ_{22}. With use of eq 4, rough approximation of the σ_{22}'s for peptide carbonyls could be combined with assignment techniques such as $^{13}C/^{15}N$ double labeling[39] or site-specific mutations[40] to create a list of approximate shift tensors. We have used the data of Kainosho et al.[39] to estimate σ_{22} for several peptide carbonyl carbons in *Streptomyces subtilisin* inhibitor. These values range from −78.4 to −50.8 ppm (relative to liquid benzene), with several values falling significantly downfield of the values observed in peptides **1-4**. Although no direct correlation between isotropic carbonyl shifts (and therefore predicted σ_{22}'s) and protein secondary structure is apparent,[39] there may be other structure-dependent effects, such as hydrogen bond strengths, which determine peptide carbonyl σ_{22}'s.

Conclusion

The data presented here demonstrate that there is significant variation in both the magnitude and orientation of carbonyl ^{13}C chemical shift tensors of several synthetic peptides. Most of this variation is due to interactions in the crystal lattice and not to purely intramolecular interactions. As previously observed with other carbonyl ^{13}C chemical shift tensors, σ_{22} is the most variable of the three principal values and σ_{33} is perpendicular to the peptide plane, to within a few degrees.

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Registry No. **1**, 109929-74-0; **1** (ethyl ester), 109929-78-4; **2**, 109929-75-1; **2** (ethyl ester), 109929-80-8; **3**, 88815-61-6; **3** (ethyl ester, free base), 109929-81-9; **4**, 109929-29-5; **4** (ethyl ester), 109929-82-0; **5**, 109929-76-2; **5** (ethyl ester), 109929-83-1; **6**, 543-24-8; ethyl [^{15}N]-DL-alanate hydrochloride, 109929-77-3; ethyl [^{15}N]-DL-tyrosinate, 109929-79-5; benzyloxycarbonyl-[*1*-^{13}C]-glycine, 67739-37-1; [^{15}N]-glycine ethyl ester hydrochloride, 58420-99-8; ethyl [^{15}N]-L-phenylalanate, 94601-37-3.

The Amide ^{15}N Chemical Shift Tensors of Four Peptides Determined from ^{13}C Dipole-Coupled Chemical Shift Powder Patterns

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Abstract: The ^{15}N chemical shift tensors of a homologous series of peptides of the form *N*-acetyl[1-^{13}C]glycyl[^{15}N]-X-amide (X = glycine, alanine, and tyrosine) and the unprotected dipeptide [1-^{13}C]glycyl[^{15}N]glycine hydrochloride have been determined from ^{13}C dipole-coupled ^{15}N powder patterns. It was found that the shift tensor principal values differ greatly while their molecular orientation does not. The common shift tensor orientation places σ_{22} perpendicular to the peptide plane, and σ_{33} at a 99° angle with respect to the C-N bond. The orientations of σ_{11} and σ_{22} were previously unknown for ^{15}N chemical shift tensors of amides. Comparison of magic angle spinning (MAS) spectra with solution spectra shows significantly different solid and solution isotropic chemical shifts for several of the peptides studied, demonstrating that at least part of the variation in principal values is due to lattice effects. This conclusion is borne out by the MAS spectrum of *N*-acetyl[1-^{13}C]glycl-[^{15}N]phenylalaninamide, which shows at least three peaks corresponding to different lattice environments.

Solid-state ^{15}N nuclear magnetic resonance (NMR) has recently proven very useful in the structural, chemical, and dynamic characterization of proteins.[2-8] In many of these studies the chemical shift anisotropy of the ^{15}N nucleus played a crucial role, and in several cases a knowledge of the chemical shift tensor of the ^{15}N was critical to the interpretation of the spectra.[3,6-8] Unfortunately, relatively few ^{15}N chemical shift tensors have been determined,[9,10] thus limiting the accuracy of the structural in-

Table I. Peptide Molecules Used in This Study

	A-NHCH$_2$^{13}C(O)^{15}NC(R)HC(O)-B			
	A	R	B	abbreviation
1	CH_3CO	DL-CH_3(DL-Ala)	NH_2	AcGlyAlaNH_2
2	CH_3CO	DL-$CH_2C_6H_4OH$(DL-Tyr)	NH_2	AcGlyTyrNH_2
3	H	H(Gly)	OH	GlyGly·HCl
4	CH_3CO	H(Gly)	NH_2	AcGlyGlyNH_2
5	CH_3CO	L-$CH_2C_6H_5$(L-Phe)	NH_2	AcGlyPheNH_2

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(2) (a) MacKenzie, N. E.; Fagerness, P. E.; Scott, A. I. *J. Chem. Soc., Chem. Commun.* **1985**, 635-637. (b) Limbach, H. H.; Hennig, J.; Kendrick, R.; Yannoni, C. S. *J. Am. Chem. Soc.* **1984**, *106*, 4059-4060.

(3) Harbison, G. S.; Herzfeld, J.; Griffin, R. G. *Biochemistry* **1985**, *22*, 1-5.

(4) Huang, T. H.; Bachovchin, W. W.; Griffin, R. G.; Dobson, C. M. *Biochemistry* **1984**, *23*, 5933-5937.

(5) Munowitz, M.; Bachovchin, W. W.; Herzfeld, J.; Dobson, C. M.; Griffin, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 1192-1196.

(6) Cross, T. A.; Opella, S. J. *J. Mol. Biol.* **1982**, *159*, 543-549.

(7) Cross, T. A.; Tsang, P.; Opella S. J. *Biochemistry* **1983**, *22*, 721-726.

(8) Cross, T. A.; Opella, S. J. *J. Mol. Biol.* **1985**, *182*, 367-381.

(9) Mehring, M. *High Resolution NMR in Solids*; Springer-Verlag: New York, 1983; p 258.

(10) Harbison, G. S.; Jelenski, L. W.; Stark, R. E.; Torchia, D. A.; Herzfeld, J.; Griffin, R. G. *J. Magn. Reson.* **1984**, *60*, 79-82.

0002-7863/87/1509-5962$01.50/0