chemical shift tensor. 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.

Whatever the nature of these lattice-dependent effects, they clearly preclude the use of model compound chemical shift tensors to determine the shift tensors of peptide ^{15}N in proteins, even if the side chain composition of the model compound matches that of the protein peptide of interest. In studies that utilize peptide ^{15}N chemical shift measurements of oriented proteins to deduce protein backbone structure,[7,8] variations in the ^{15}N shift tensor such as those reported here can be extremely important. Assume, for instance, that a peptide group in such a protein is oriented with the magnetic field perpendicular to the peptide plane. Assume also that the ^{15}N shift tensor of this peptide is the same as that of AcGlyAlaNH$_2$. Under these conditions, the observed ^{15}N chemical shift would be 85.1 ppm. If this chemical shift were interpreted assuming that the tensor for GlyGly·HCl applied, the predicted orientation would be in error by 24.6°.[21] There are many circumstances when assuming the incorrect tensor would make it impossible to deduce any orientation at all.[22]

The alternative to assuming a canonical shift tensor is to determine the principal values of the ^{15}N tensor for the actual peptide group in the protein being studied. This could be accomplished by obtaining the spectrum of a motionless powder of the protein, either directly or by slow-speed MAS and fitting the powder spectrum or by analyzing the sideband intensities.[12] By labeling the adjacent amino acid with ^{13}C in the carbonyl position, the ^{15}N can be selectively detected in static[23] or MAS[11d] spectra.

Conclusion

We have shown that there are large, lattice-dependent variations in the ^{15}N chemical shift tensor principal values of several model dipeptides. However, there are no significant differences in the molecular orientations of these tensors. These conclusions have led us to suggest a new approach for the use of ^{15}N peptide chemical shift measurements to determine protein structure based on the direct measurement of the principal values of the shift tensor in the molecule of interest using $^{15}N/^{13}C$ double-labeling and selective detection.[11d,23] These measurements would greatly improve the reliability of peptide ^{15}N chemical shift anisotropy in the determination of protein structure.

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Registry No. 1, 109929-74-0; 2, 109929-75-1; 3, 88815-61-6; 4, 109929-29-5; 5, 109929-76-2.

(21) There is an infinite set of orientations that have a chemical shift of 85.1 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 $^{15}N-^{13}C$ dipolar coupling of a doubly labeled peptide oriented with its N–C bond parallel to the magnetic field would be 1300 Hz, assuming standard peptide geometries. The orientation calculated in this example corresponds to the intersection of the 85.1 ppm chemical shift and 1300 Hz dipolar coupling isochromats in the principal axis systems of the GlyGly·HCl chemical shift tensor.

(22) These cases correspond to occasions in which no intersection between the chemical shfit and dipolar isochromats (see ref 21) exists due to the fact that the incorrect shift tensor does not yield a physically reasonable chemical shift for a given orientation.

(23) Oas, T. G.; Hartzell, C. J.; Drobny, G. P.; Dahlquist, F. W. *J. Magn. Reson.*, submitted for publication.

Determination of the ^{15}N and ^{13}C Chemical Shift Tensors of L-[^{13}C]Alanyl-L-[^{15}N]alanine from the Dipole-Coupled Powder Patterns

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Abstract: The ^{13}C and ^{15}N chemical shift tensors of L-[1-^{13}C]alanyl-L-[^{15}N]alanine have been determined from the dipole-coupled powder patterns and verified with the decoupled spectra. The principal values of the ^{13}C tensor are $\sigma_{11} = -115.5$, $\sigma_{22} = -42.3$, and $\sigma_{33} = 33.5$ ppm, and the polar angles relating the $^{13}C-^{15}N$ bond to the chemical shift tensor are $\beta = 90°$ and $\alpha = -39.5°$. Although the ^{13}C powder pattern widths of AlaAla and AcGlyAlaNH$_2$ differ by only 2%, the anisotropy and asymmetry parameters differ by 10 and 25%, respectively. The principal values for the ^{15}N chemical shift tensor are $\sigma_{11} = 65.3$, $\sigma_{22} = 78.1$, and $\sigma_{33} = 215.5$ ppm, and the orientation of the $^{13}C-^{15}N$ bond to the σ_{33} axis is 106°. AlaAla and AcGlyAlaNH$_2$ show a striking difference in asymmetry parameters (0.06 vs. 0.16) and anisotropy (144 vs. 165).

We report here the ^{13}C and ^{15}N chemical shift tensors of polycrystalline L-[1-^{13}C]alanyl-L-[^{15}N]alanine (AlaAla). This is the first determination in a nonglycine dipeptide of the polar angles relating the $^{13}C-^{15}N$ bond to the ^{13}C and ^{15}N chemical shift tensors. Absolute orientation to the molecular frame is dependent on analogy to single-crystal studies which place σ_{33} of the ^{13}C chemical shift tensor perpendicular to the peptide plane[2] and σ_{33} of the ^{15}N chemical shift tensor in the peptide plane.[3] Although ^{13}C chemical shift tensors have been studied extensively,[4] the only complete acyl ^{13}C tensor determined in an amide group is reported for the dipeptide GlyGly·HCl.[2] Studies of ^{15}N chemical shift

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tensors are less abundant, and the only reported amide ^{15}N chemical shift tensor is that in GlyGly·HCl.[3] It is, therefore, timely to investigate the principal values and orientation of other peptide ^{13}C and ^{15}N chemical shift tensors.

The ^{13}C chemical shift tensor is a powerful tool for studying motion in amino acids[5] due to the change in powder pattern shape as a result of rotational or flip averaging. Studies of motion in proteins rely on a knowledge of the static ^{13}C powder pattern width and principal values.[6,7] Choice of an appropriate static model is crucial to correct interpretation of the isotopically labeled protein spectra. We have recently reported[8] that both the principal values and the orientation of the ^{13}C carbonyl chemical shift tensor to the peptide bond vary with a change of substituent X in the homologous series of GlyX dipeptides, *N*-acetyl[1-^{13}C]glycyl-[^{15}N]-X-amide (X = glycine, alanine, tyrosine). Likewise, a recent study of a variety of [1-^{13}C]glycine-containing polypeptides[9] reported a variation in the principal values of the chemical shift tensor depending on the neighboring amino acids and the secondary structure of the polypeptide. To further investigate the variation in the ^{13}C carbonyl shift tensor with environment, we have determined both the principal axis values and the orientation of the ^{13}C carbonyl chemical shift tensor of L-[1-^{13}C]alanyl-L-[^{15}N]alanine.

We have also reported previously[10] that the principal values of the ^{15}N chemical shift tensor vary with a change of substituent X in the same homologous series of GlyX dipeptides. Peculiarities evident in that study prompted us to study a dipeptide differing in the carbonyl substituent. In particular, powder pattern simulations remained insensitive to rotations about σ_{33} by ±5° (α = 0 ± 5°), and the angle between σ_{33} and the C–N bond was identical (β = 99 ± 2°), within experimental error, for all the GlyX dipeptides. This behavior is unlike that of ^{13}C carbonyl shift tensors in the GlyX dipeptides[8] which show a variation of 12° in the angle α.

Studies of peptide plane orientation and motion in proteins rely on a knowledge of ^{15}N chemical shift powder pattern widths and tensor orientation.[2,3,11] But despite these important applications of nitrogen chemical shift tensors, the principal values and orientations of only a few amide chemical shift tensors have been reported and only for GlyX dipeptides.[3,10] This study of AlaAla provides another comparison for determining whether all peptide ^{15}N chemical shift tensors have the same orientation relative to the ^{13}C–^{15}N bond.

The principal values and polar angles were determined from a powder pattern, taking advantage of the sensitive dependence of the powder line shape on the orientation of the dipole tensor within the chemical shift principal axis system. This method makes accessible chemical shift tensors in materials which do not readily yield single crystals such as large proteins, particularly membrane-bound proteins, and liquid crystals. The powder patterns were analyzed using a gradient least-squares fitting procedure as described previously.[8]

Experimental Section

Powdered, zwitterionic L-[1-^{13}C]alanyl-L-[^{15}N]alanine (AlaAla) was purchased from MSD Isotopes (St. Louis). Both ^{13}C and ^{15}N enrichment was 99%. Spectra were obtained on a 0.15-g sample. A S/N = 100 was obtained after 2880 scans for the ^{15}N-coupled ^{13}C spectrum, while 3600 scans gave a S/N = 26 for the ^{15}N-decoupled ^{13}C spectrum. A S/N = 100 was obtained after 2560 scans for the ^{13}C-coupled ^{15}N spectrum, while 4006 scans gave a S/N = 20 for the ^{13}C-decoupled ^{15}N spectrum.

Cross-polarization was used to transfer polarization from ^{1}H to ^{13}C or ^{15}N. The mixing time was 5 ms and the B_1 rf fields during mixing were 42 G for ^{13}C and 11 G for ^{1}H (62 and 21 W, respectively) and 41 G for ^{15}N and 4 G for ^{1}H (93 and 4 W, respectively). To eliminate transient noise, a π echo pulse with τ = 50 μs was utilized before acquisition. All free induction decays (fid) were recorded in the presence of a 20-G ^{1}H-decoupling field (83.5 kHz, 62 W). The ^{15}N-decoupled ^{13}C spectra were obtained by applying a waltz decoupling sequence[12] of 50-μs pulses at 15 W (a decoupling field of 12 G, 5 kHz) during acquisition. The ^{13}C-decoupled spectra were obtained by applying an 11-G decoupling field during acquisition.

Spectra were obtained on a Bruker CXP-200 spectrometer with a 4.7-T magnet. The probe, constructed in this lab, utilized a single coil, 24 mm × 6 mm, triply tuned for proton (200 MHz), carbon-13 (50.3 MHz), and nitrogen-15 (20.27 MHz). A 50.3-MHz trap was placed in the nitrogen-15 circuit as a high impedence to 50.3-MHz rf. Reference spectra of solid hexamethylbenzene (−111.5 ppm relative to liquid benzene) or solid [^{15}N]ammonium chloride (38.5 ppm relative to $^{15}NH_3$ at −50 °C) were taken before each dipeptide study.

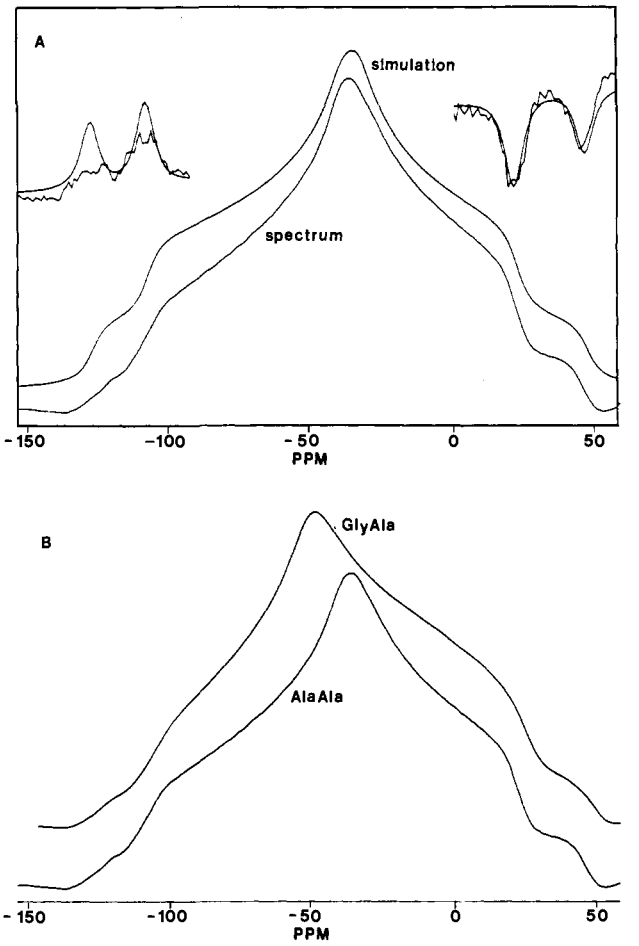

Figure 1. (A) Comparison of the ^{13}C spectrum of AlaAla (lower curve) with the best-fit simulation (upper curve). (B) Comparison of the ^{13}C spectra of AlaAla (lower curve) and GlyAla (upper curve).

Results and Discussion

^{13}C Study. Figure 1A compares the ^{15}N-coupled ^{13}C powder pattern obtained for AlaAla with the simulated spectrum resulting from the best-fit parameters. Fitting the first derivative proved most sensitive to the inflection points at the edges of the spectra. Insets display the first derivative of the spectrum and the first derivative fit results. The principal values of the ^{13}C shift tensor of AlaAla (in ppm from liquid benzene) are:

$$\sigma_{11}\ -115.5 \qquad \sigma_{22}\ -42.3 \qquad \sigma_{33}\ 33.5$$

The polar angles are β = 90.2° and α = −39.5°, where β is rotation about σ_{22} and α is subsequent rotation about σ_{33}.

These principal values agree closely with the values read from the ^{15}N-decoupled spectrum: −115.6, −41.2, and 33.6 ppm. The

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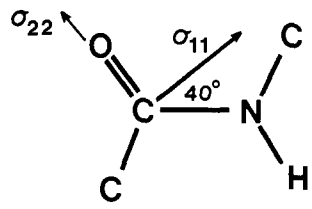

Figure 2. Orientation of the ^{13}C chemical shift tensor in the plane of the peptide bond assuming σ_{33} is perpendicular to the peptide plane. X-ray data[16] give an O–C–N angle of 125.9°.

Table I. Alanine and Glycine ^{13}C Carbonyl Chemical Shift Tensors, Anisotropy, and Asymmetry Parameters

molecule	principal values (ppm) σ_{11}	σ_{22}	σ_{33}	$2/3\delta^a$	η^b	angle α^c
AlaAla	−115.5	−42.3	33.5	112	0.44	−39.5
$AcGlyAlaNH_2$	−113.6	−56.4	38.5	124	0.31	−36.6
$AcGlyTyrNH_2$	−114.0	−37.0	33.2	109	0.47	−40.7
GlyGly·HCl	−115.3	−48.7	39.4	121	0.37	−46.6
$AcGlyGlyNH_2$	−114.5	−55.7	37.3	122	0.32	−34.5

[a] Anisotropy:[15] $2/3\delta = \sigma_{33} - {}^1/_2(\sigma_{11} + \sigma_{22})$. [b] Asymmetry parameter:[15] $\eta = (\sigma_{22} - \sigma_{11})/\delta$. [c] Angle between σ_{11} and ^{13}C-^{15}N bond, deg

best-fit value of β agrees with previous results from single-crystal studies of glycine[13] and alanine[14] showing σ_{33} to be nearly perpendicular (β = 87°) to the O–C–O plane of the carboxylic acid. Assuming this orientation of σ_{33}, Figure 2 shows the orientation of σ_{11} and σ_{22} in the peptide plane. Note that σ_{22} lies 3.6° from the C=O bond. The value of α determined for AlaAla falls in the middle of the range presented by the GlyX dipeptides, as compared in Table I. For simulation, a dipolar coupling of 1260.3 Hz was used in agreement with the C–N bond length of 1.34 Å given by X-ray crystallography data.[16]

The principal values of the ^{13}C chemical shift tensor of AlaAla lie within the range of values obtained for the GlyX dipeptides which are listed in Table I. The powder pattern widths of AlaAla and GlyAla differ by 3 ppm (2%) which equals the standard deviation of the GlyX dipeptides. The difference in anisotropy and asymmetry parameters for these two dipeptides is more striking, 10 and 25%, respectively, while the standard deviation among the GlyX dipeptides is 6 and 9% for these parameters. This difference in axial symmetry is obvious in the comparison of the AlaAla and GlyAla spectra shown in Figure 1B. Such differences in powder pattern widths and asymmetry parameters are of consequence since these values are of a magnitude that might be misinterpreted as motion in an improperly applied model. Simulations of rotational diffusion and six-fold jump models of nonaxially symmetric powder spectra show that the rapid motion limit is axially symmetric.[17,18]

Pines et al.[19] have shown that σ_{22} rotates away from the carbonyl bond as the structure of the ^{13}C-containing molecule changes from a ketone to an ester to an unsymmetrical, then a symmetrical carboxyl group. The orientation of the tensor in the dipeptides is much closer to that of a ketone than to that of a symmetrical carboxyl group. The orientation of σ_{22} at 3.2° from the carbonyl bond in AlaAla differs considerably from the orientation in a carboxylic acid[4] of nearly 30°. Likewise, σ_{11} is closer to the C–N bond in a peptide than to the C–O bond of an acid. Naito et al.[14] have previously reported principal values for the carbonyl chemical shift tensor of single-crystal alanine. These values (−114, −55, 21.8 ppm) describe a tensor differing little in anisotropy ($2/3\delta$ = 106.3) and asymmetry parameter (η = 0.37) from the peptide carbonyl studied here. The anisotropy and asymmetry parameter of [^{13}C]alanine are closer to the values for AlaAla than are the GlyAla values.

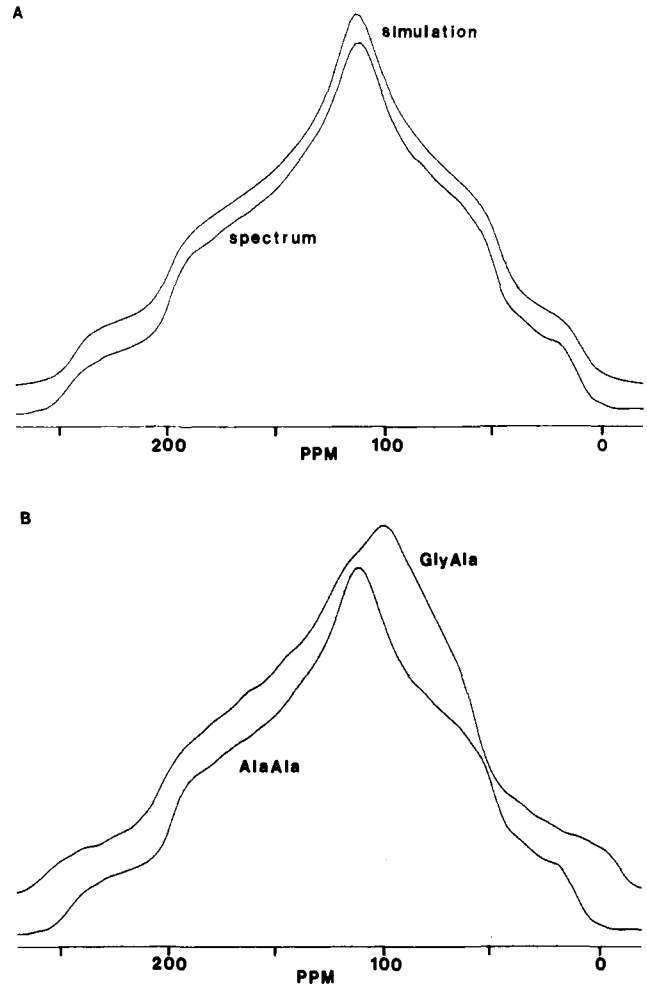

Figure 3. (A) Comparison of the ^{15}N spectrum of AlaAla (lower curve) with the best-fit simulation (upper curve). (B) Comparison of the ^{15}N spectra of AlaAla (lower curve) and GlyAla (upper curve).

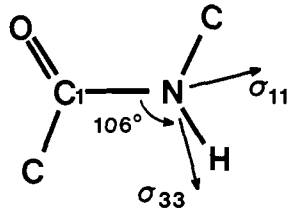

Figure 4. Orientation of the ^{15}N chemical shift tensor in the plane of the peptide bond. The angle[16] C(1)–N–H is 117.6°.

^{15}N Study. The experimental ^{15}N spectrum is compared to the best-fit simulation in Figure 3A. The best-fit determination of the principal values of the AlaAla ^{15}N shift tensor (in ppm from liquid $^{15}NH_3$) is:

$$\sigma_{11}\ 65.3 \qquad \sigma_{22}\ 78.1 \qquad \sigma_{33}\ 215.5$$

and the orientation is given by β = 106° and α = 5°, where β is the angle formed by σ_{33} with the ^{13}C–^{15}N bond and α is the projection of ^{13}C–^{15}N on the σ_{11}–σ_{22} plane. We see changes of less than 2 ppm in the principal values and a change of only 3° in β when α is changed from 2 to 10°, indicating that the line

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Table II. ^{15}N Dipeptide Chemical Shift Tensors, Anisotropy, and Asymmetry Parameters

molecule	principal values (ppm) σ_{11}	σ_{22}	σ_{33}	$2/3\delta^a$	η^b	angle β^c
AlaAla	65.3	78.1	215.5	144	0.06	106
$AcGlyAlaNH_2$	44.6	85.1	229.4	165	0.16	100
$AcGlyTyrNH_2$	52.1	77.1	209.3	145	0.12	98
GlyGly·HCl	57.3	59.8	210.0	152	0.01	99
$AcGlyGlyNH_2$	40.7	64.2	210.6	158	0.10	100

[a] Anisotropy:[15] $2/3\delta = \sigma_{33} - {}^1/_2(\sigma_{11} + \sigma_{22})$. [b] Asymmetry parameter:[15] $\eta = (\sigma_{22} - \sigma_{11})/\delta$. [c] Angle between σ_{33} and ^{13}C–^{15}N bond, deg.

shape is insensitive to small rotations about σ_{33}. The refined best-fit values of $\beta = 106°$ and $\alpha = 5°$ were obtained only after careful analysis of ^{13}C-coupled, ^{1}H dipole-modulated ^{15}N spectra[20] to determine the orientation of the ^{15}N–H bond in the principal axis system of the chemical shift tensor. The report of this study is forthcoming.[21] The simulations are fit with a dipolar coupling constant of 1260 Hz which gives the length of the C–N bond as 1.34 Å, in agreement with X-ray data.[16] This value of the dipolar coupling constant is the same as that determined for the ^{15}N-coupled ^{13}C chemical shift powder pattern of AlaAla. Based on a single-crystal study of GlyGly·HCl,[4] the rotation through β is assumed to take place in the peptide plane. A suggested orientation of the ^{15}N chemical shift tensor in the peptide plane is shown in Figure 4.

A comparison of the principal values, the chemical shift anisotropy, and the asymmetry parameter for AlaAla with those for GlyX is given in Table II. Most interesting is the large difference in the anisotropy (144 vs. 165) and asymmetry parameters (0.06 vs. 0.16) of AlaAla and GlyAla, respectively. The contrast is evident in the comparison of the spectra of AlaAla and GlyAla shown in Figure 3B. Both the asymmetry parameter and the anisotropy of AlaAla are smaller than the values of the end-protected GlyX dipeptides. The near-symmetry of the ^{15}N chemical shift tensor in AlaAla was evident in the ^{13}C-decoupled spectrum (data not shown).

These differences in the chemical shielding tensor are most likely related to differences in local structure and symmetry in the dipeptides studied. To predict the structural differences responsible for these spectra would require X-ray studies on the series of dipeptides. The past studies of ^{13}C and ^{15}N chemical shift tensors have included attempts to correlate the observed shift tensors to the molecular orbitals involved[22] and attempts to apply ab initio calculations.[23] Rationalization of the tensor differences reported in this study would require such methods and is outside the scope of this work.

Toward the goal of unambiguously determining the chemical shift tensor orientation from powder spectra, we have submitted a manuscript describing the use of two dipolar interactions to determine the orientation of the ^{15}N chemical shift tensor in the peptide plane without reference to single-crystal studies.[21]

Acknowledgment. The authors thank Dr. Thomas Pratum for lending his instrumentation expertise. This work was funded by PHS GM32681-03.

Note Added in Proof. While this manuscript was in press, a study was published of the ^{15}N chemical shift tensor of the imide nitrogen in alanyl-proline.[24] A comparison of the orientation of the deshielded principal element (σ_{11} in that paper) to the value of β determined herein shows a difference of 4°. The more striking difference is in the asymmetry parameter which is 0.46 for the imide nitrogen.

Registry No. L-[1-^{13}C]alanyl-L-[^{15}N]alanine, 109929-26-2; *N*-acetyl-[1-^{13}C]glycyl-L-[^{15}N]alanineamide, 109929-27-3; *N*-acetyl[1-^{13}C]-glycyl-L-[^{15}N]tyrosineamide, 109929-28-4; [1-^{13}C]glycyl[^{15}N]glycine hydrochloride, 88815-61-6; *N*-acetyl[1-^{13}C]glycyl[^{15}N]glycineamide, 109929-29-5.

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