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HYDROGEN-BONDING EFFECT ON ^{15}N NMR CHEMICAL SHIFTS OF THE GLYCINE RESIDUE OF OLIGOPEPTIDES IN THE SOLID STATE AS STUDIED BY HIGH-RESOLUTION SOLID-STATE NMR SPECTROSCOPY

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ABSTRACT

High-resolution ^{15}N NMR spectra of a variety of solid oligopeptides (X-Gly-Gly) containing glycine residues have been measured, of which the crystal structures had already been determined by X-ray diffraction The experimental ^{15}N chemical shifts of the glycine residues were plotted against the N···O hydrogen bond length and the N–H bond length in the $\rangle$C=O···H–N$\langle$ type hydrogen bond form, respectively It was found that the decrease of the N–H bond length leads to a linear increase in ^{15}N shielding, but there is no clear relationship between the ^{15}N chemical shifts and the N···O separation Further, ^{15}N chemical shift calculations were carried out using a model compound, by the FPT-INDO method, in order to further understand the nature of the hydrogen bond The calculated results reasonably explain the experimental ones

INTRODUCTION

Recently, high-resolution ^{15}N NMR spectroscopy in the solid state has been increasingly applied to the investigation of polypeptides, proteins and biopolymers [1–12]. In a previous paper [13], we demonstrated that the isotropic ^{15}N chemical shifts of a number of homopolypeptides in the solid state, as determined by the cross polarization–magic angle spinning (CP-MAS) method, are significantly displaced according to their particular conformations such as the α-helix and β-sheet forms. Moreover, it was found that the ^{15}N chemical shift difference between the α-helix and β-sheet forms for such homopolypeptides in the solid state varies by as much as 1.2–10.0 ppm, depending mainly

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on the nature of the amino acid residue [13] Such a large chemical shift difference may come from changes in the hydrogen bond through changes in the dihedral angles (ϕ, ψ).

We have also measured ^{13}C CP-MAS NMR spectra of some oligopeptides containing glycine residues in the solid state [14], in order to obtain information about the relationship between the carbonyl carbon chemical shift and hydrogen bond length. It was found that the ^{13}C signals of the carbonyl carbons in the $>C=O\cdots H-N<$ type hydrogen bond form are deshielded with a decrease in the hydrogen bond length but those in the $>C=O\cdots H-N^{+}<$ type hydrogen bond form are shielded with a decrease in the hydrogen bond length Quantum chemical calculations of the ^{13}C shieldings for model compounds were performed and found to reproduce reasonably the experimental results, taking into account the hydrogen bond and conformational effects [14]. In this work, in order to obtain and accumulate further knowledge of the hydrogen bonding in peptides, we attempt to measure ^{15}N CP-MAS NMR spectra of the oligopeptides containing glycine residues in the solid state and to clarify the origin of the relationship between the ^{15}N chemical shift and the manner of the hydrogen bond. Further, in an attempt to obtain a deep insight into the nature of the hydrogen bond, we calculate the ^{15}N shieldings and tensor components of the glycine amide nitrogens by employing quantum-chemical methods.

EXPERIMENTAL SECTION

Materials

A series of oligopeptides containing glycine residues, except for sarcosylglycylglycine (Sar-Gly-Gly), were purchased from Sigma Co. and were recrystallized according to the same procedures as those used in the X-ray diffraction studies on them. Sar-Gly-Gly was synthesized by stepwise elongation of *N*-hydroxysuccinimide active esters and amino acids [15]. The N-terminal of the active ester was protected by the *o*-nitrophenylsulfenyl (Nps-) group. This sample was purified and recrystallized from aqueous solution. Glycylglycine nitrate (Gly-Gly·HNO_3) was obtained by slow evaporation from an equimolar mixture of glycylglycine and nitric acid in water. Glycylglycine monohydrochloride monohydrate (Gly-Gly·HCl·H_2O) was obtained by slow evaporation from an equimolar mixture of glycylglycine and hydrogen chloride in water.

Measurements

^{15}N CP-MAS NMR spectra were recorded at room temperature and 27.25 MHz with a JEOL GSX-270 NMR spectrometer equipped with a CP-MAS accessory. Field strength of the ^{1}H decoupling was 1.2 mT. The contact time was 5 ms, and the repetition time was 10 s. Spectral width and data points were

20 kHz and 8k, respectively. Samples were placed in a cylindrical rotor and spun at 4–5 kHz. Spectra were usually accumulated 100–600 times to achieve a reasonable signal-to-noise ratio. ^{15}N chemical shifts were calibrated indirectly through external glycine-^{15}N ($\delta = 11.59$ ppm; line width = 17 Hz) relative to saturated $^{15}NH_4NO_3$ ($\delta = 0$ ppm) solution in H_2O.

Theoretical calculation

We employed the finite perturbation theory (FPT) within the INDO framework for calculating the ^{15}N shieldings. The FPT INDO theory has the advantage of permitting the calculation of the paramagnetic term without requiring the explicit wavefunctions of the excited states and the one-electron excitation energies, which are difficult to obtain with high accuracy by the usual semiempirical MO approximations. This approach reproduces reasonably the experimental ^{13}C chemical shifts of L-alanine residues in peptides [16]. According to the FPT INDO framework [17–19], $\sigma^{d}_{\alpha\beta}(A)$ (diamagnetic term) and $\sigma^{p}_{\alpha\beta}(A)$ (paramagnetic term) are expressed by

$$\sigma^{d}_{\alpha\beta} = \frac{e^2}{2mc^2} \sum_{\mu} \sum_{\nu} P\mu\nu(0) \left\langle \chi_\mu \left| \frac{\gamma_\nu \gamma_A \delta_{\alpha\beta} - \mathbf{r}_{\nu A} \mathbf{r}_{A\beta}}{|\gamma_A|^3} \right| \chi_\nu \right\rangle$$

$$\sigma^{p}_{\alpha\beta} = -\frac{eh}{mc} \imath \sum_{\mu} \sum_{\nu} \left(\frac{\partial P_{\mu\nu}(B_\alpha)}{\partial B_\alpha} \right)_{B=0} \left\langle \chi_\mu \left| \frac{(\gamma_A \times \nabla)_\beta}{|\gamma_A|^3} \right| \chi_\nu \right\rangle$$

$$\alpha, \beta = x, y, z$$

where the gauge origin of the vector potential is set at the position of nucleus A The vectors $\mathbf{r}_\nu$ and $\mathbf{r}_A$ are the position vectors of the electron considered from the nucleus of the atom containing the atomic orbital (AO) χ_ν and from the nucleus A, respectively. $P_{\mu\nu}(B)$ and $P_{\mu\nu}(0)$ are the elements of the density matrix with and without the perturbation due to the magnetic field, B, respectively. The ^{15}N shielding calculation was carried out by a similar procedure to that reported previously for a ^{13}C shielding calculation [13]. In the calculation, we adopted *N*-acetyl-*N'*-methylglycine amide as a model compound. The bond lengths and bond angles proposed by Momany et al. [20] were used. A HITAC M780H computer at the Computer Center of the Tokyo Institute of Technology was used for the calculation The N–H bond length and N···O hydrogen bond length, optimized by an ab initio STO-3G MO calculation, were determined for *N*-methylacetamide as a model compound. The calculations were performed on a HITAC M680H computer at the Computer Center of the Institute for Moleculer Science, Okazaki.

RESULTS AND DISCUSSION

^{15}N NMR chemical shifts of glycine amide nitrogens of peptides in the solid state

A 27.25 MHz ^{15}N CP-MAS NMR spectrum of L-alanylglycylglycine (L-Ala-Gly-Gly) in the solid state is shown as a typical example in Fig. 1. ^{15}N CP-MAS spectra of the remaining samples were also obtained with similar resolutions. Signals were also assigned with reference to previous ^{15}N CP-MAS NMR and solution state ^{15}N NMR data [21].

We use selected glycylglycine (X-Gly-Gly) sequence oligopeptides in this work. It is well known that the ^{15}N chemical shifts of peptides vary with the amino acid sequence [22]. For example, ^{15}N chemical shift of the glycine residue depends upon the amino acid residue linked to the N-terminal of the glycine residue. Consequently, we use the glycylglycine sequence oligopeptides, in order to neglect the sequence-dependent ^{15}N chemical shifts.

All the ^{15}N chemical shift values of oligopeptides determined from the observed spectra are tabulated in Table 1, together with the geometrical parameters obtained by X-ray diffraction studies. Some of the geometrical parameters were calculated by using the unit cell parameters and fractional coordinates given in the literature [23–31]. The ^{15}N chemical shifts and the geometrical parameters of tert-butyloxycarbonyl-glycylglycylglycine-benzylester (Boc-Gly-Gly-Gly-OBzl) are inferred from the results of Hiyama et al. [31].

Figure 2 shows the plot of the observed ^{15}N chemical shifts of Gly NH against the hydrogen bond length N···O ($R_{N\ O}$). However, it is found that there is no clear relationship between $R_{N\ O}$ and ^{15}N chemical shifts. This is different

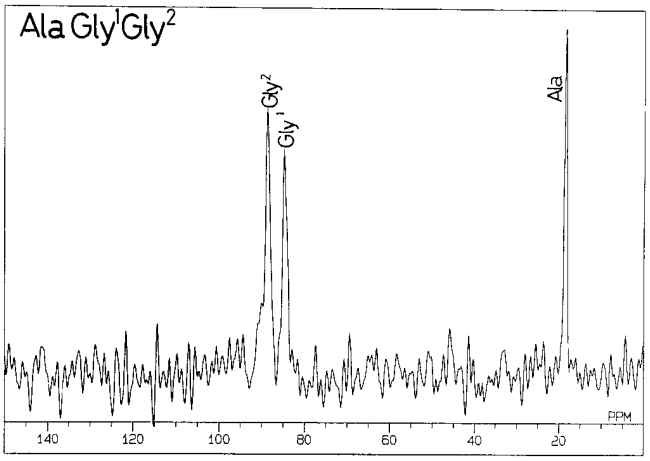

Fig 1 A typical 27 25 MHz ^{15}N CP-MAS NMR spectrum of L-alanylglycylglycine in the crystalline state

TABLE 1

^{15}N chemical shift of glycine residue amide nitrogens for oligopeptides containing glycine as determined by ^{15}N CP-MAS NMR (± 0.2 ppm from $^{15}NH_4NO_3$) and the geometrical parameters of X-Gly-Gly peptides

Sample	^{15}N chemical shift[a], δ (ppm)	Geometrical parameters						
		Dihedral angle (deg)		N···O (Å)	H···O (Å)	N-H (Å)	N-H ···O (deg)	Ref
		ϕ	ψ					
GlyGly*OH	99.95	157.1	10.7	2.94	1.97	1.02	158	23
GlyGly*·H_2O HCl	90.95	−79.6	3.8	3.30[b]	2.30	0.79	162	24
GlyGly*OH·HNO_3	89.71	165.6	176.9	3.12	2.38	0.76	165	25
ValGlyGly*OH	94.43	−146.6	−4.3	3.05	2.19	0.93	152	26
ProGlyGly*OH	89.44	−110.5	175.1	2.89	2.07	0.85	165	27
AlaGlyGly*OH	88.55	170.9	175.2	3.00	2.19	0.84	160	28
SarGlyGly*OH	89.00	−85.4	−177.5	3.06	2.23	0.85	167	29
TyrGlyGly*OH	92.30	−103.9	−152.6	2.88	2.13	0.86	144	30
BocGlyGlyGly*OBzl	92.75[c]	−77.8	−178.4	2.92	2.10	0.92	157	31

[a]These values are for the Gly residues with an asterisk [b]The glycine residue participates in an H ·Cl type hydrogen bond [c]This value is converted to the $^{15}NH_4NO_3$ reference from ref 31

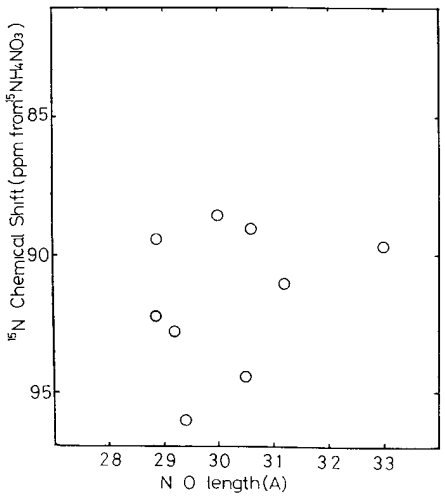

Fig 2 Plots of the observed ^{15}N shielding of oligopeptides in the crystalline state against the N···O hydrogen bond length

from the case of ^{13}C chemical shifts of the carbonyl carbons associated with the hydrogen bond which we reported previously [14] where the ^{13}C signals of the carbonyl carbons are linearly deshielded with a decrease in $R_{N\ O}$.

Figure 3 shows the plot of the observed ^{15}N chemical shifts ($\delta_{obs}{}^{N}$) of the

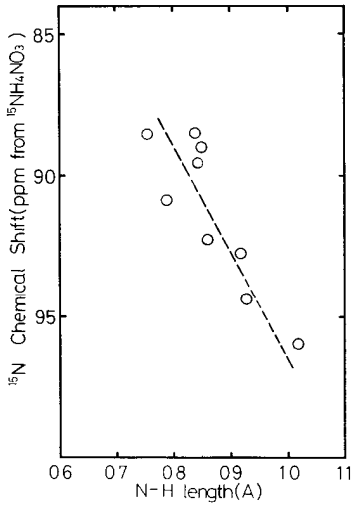

Fig 3 Plots of the observed ^{15}N shielding of oligopeptides in the crystalline state against the N-H bond length

glycine residue in X-Gly-Gly against the N–H bond length (R_{N-H}) associated with the hydrogen bond. It is found that there is a clear relationship between these parameters and the decrease of R_{N-H} leads to a linear increase in shielding. The expression for this relationship is

$$\delta_{obs}{}^{N} = 39\ 32R_{N-H} + 57.73$$

Such a trend is very different from that obtained from ^{13}C NMR. Amide ^{15}N chemical shifts are closely related to the length of the N–H bond but are not related to the N···O hydrogen bond length. This implies that the ^{15}N chemical shift value gives useful information about the N–H bond length in the hydrogen bond. It seems that the hydrogen bond angle ($\angle$ N–H···O) is also related to the ^{15}N chemical shift

Ab initio MO calculation relationship between the N–H bond length and the N···O hydrogen bond length

The structure of the two hydrogen-bonded *N*-methyl acetamides used as a model system is shown in Fig. 4. At first, the geometrical parameters of an *N*-methyl acetamide molecule were optimized using the ab initio STO-3G MO method Next, for two hydrogen-bonded *N*-methyl acetamides, the bond length N–H was optimized as a function of the hydrogen bond length N···O Figure 5 shows the relationship between the minimized bond length N–H and the hydrogen bond length N···O, as determined by ab initio MO calculations

It is shown that at $R_{N\ O} < 2.97$ Å, an increase of $R_{N\ O}$ leads to an increase

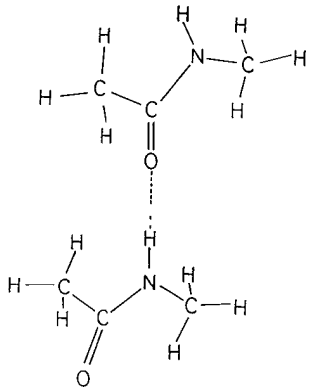

Fig 4 Molecular structure of the two hydrogen-bonded *N*-methylacetamides used as model compounds

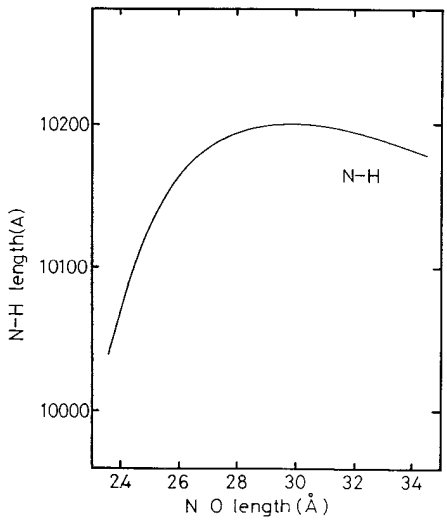

Fig 5 Plots of the calculated N-H bond length against the hydrogen bond length (N·· O) obtained using the ab initio STO-3G MO method

of $R_{N\text{-}H}$. However, at $R_{N\ O} > 2.97$ Å, an increase of $R_{N\ O}$ leads to a decrease of $R_{N\text{-}H}$. In the samples used in this work, the $R_{N\ O}$ values are between 2.85 and 3.30 Å. Therefore, it can be said that the $R_{N\text{-}H}$ values decrease with an increase of $R_{N\ O}$.

On the other hand, X-ray diffraction studies, have shown that the $R_{N\text{-}H}$ values decrease with an increase of the $R_{N\ O}$ values [32] From the above results, it can be said that there is an apparent relationship between the hydrogen bond length $R_{N\ O}$ and the bond length $R_{N\text{-}H}$.

^{15}N shielding calculations

Figures 6 and 7 show the calculated isotropic shieldings (σ_{iso}) and the paramagnetic terms of the tensor components (σ_{11}, σ_{22}, σ_{33}, from downfield to upfield) of Gly NH using the model compound *N*-acetyl-*N'* methylglycine (Fig. 8). The calculated values are all expressed in parts per million (ppm) with an opposite sign to those in Table 1. Note that the negative sign for the calculated

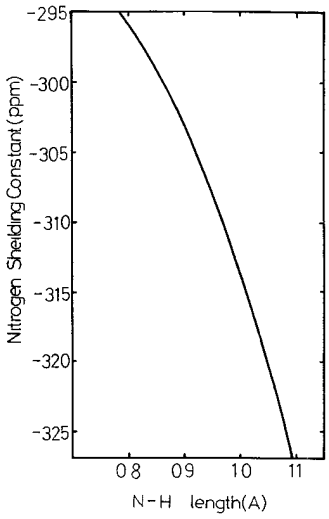

Fig 6 Plots of the calculated ^{15}N shielding against the N-H bond length from the FPT INDO method

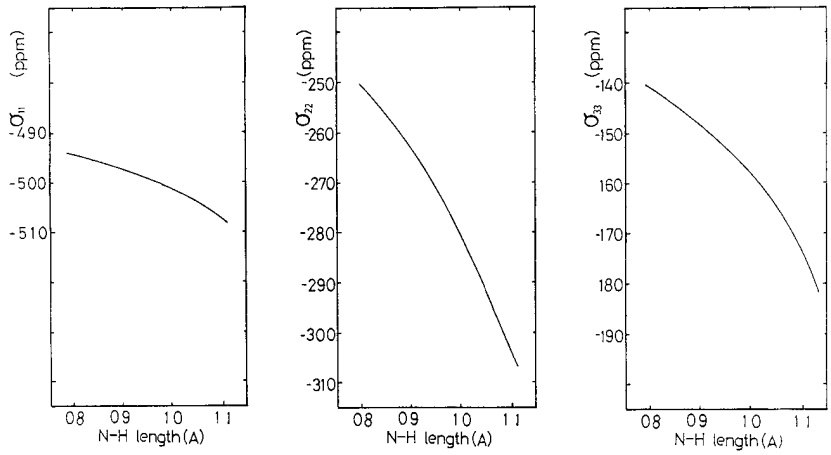

Fig 7 Plots of the calculated ^{15}N shielding tensor components against the N-H bond length from the FPT INDO method

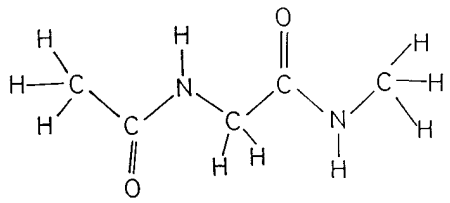

Fig 8 Molecular structure of *N*-acetyl-*N'*-methylglycine amide used as the model compound

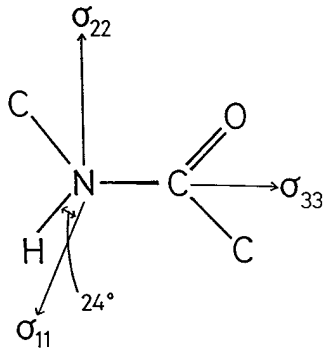

Fig 9 Orientation of the principal axes of the ^{15}N shielding tensors of the glycine residue amide nitrogen as determined in the literature [31]

shielding denotes deshielding, which is similar to the positive sign of the experimental chemical shift values. A shielding value, or tensor component, is usually represented as the sum of the diamagnetic and the paramagnetic terms. However, the anisotropic behaviour of the shielding tensor can be predominantly explained by the paramagnetic term, since the diamagnetic term is isotropic.

Figure 6 shows the R_{N-H} dependence of the calculated isotropic ^{15}N shielding (σ_{iso}) of Gly NH. It is shown that a decrease of R_{N-H} leads to a very large increase of σ_{iso}; for example, a decrease of 0.4 Å in R_{N-H} leads to a calculated shielding increase of about 30 ppm. This agrees with the observed results. This implies that the observed shielding increase is a consequence of changes in the electronic structure through a decrease of the N–H bond length. Figure 7 shows the R_{N-H} dependence of the calculated principal values of the ^{15}N shielding of Gly NH. It is shown that an decrease of R_{N-H} leads to an increase in shielding of σ_{11}, σ_{22} and σ_{33}, respectively. The magnitudes for changes of the shielding occur in the order $\sigma_{22} > \sigma_{33} > \sigma_{11}$. The direction of the principal axes of the Gly NH ^{15}N shielding tensor components, as determined by the NMR study of a Boc-Gly-Gly-Gly-OBzl single crystal [31], are shown in Fig. 9. The σ_{11} component lies approximately along the N–H bond, and the σ_{33} component lies approximately along the N–C′ bond. The σ_{22} component is aligned in the di-

rection perpendicular to the peptide plane. From this, it can be said that the σ_{22} and σ_{33} components, rather than the σ_{11} component, are sensitive to R_{N-H} changes. This may be due to the fact that the σ_{22} component lies approximately along the direction of the nitrogen lone-pair electrons and the electron density is very high in this direction. The σ_{33} component lies approximately along the N–C′ bond into which lone-pair electrons of the nitrogen atom transfer, and so the bond order becomes very high. Consequently, the σ_{22} and σ_{33} components are sensitive to changes in R_{N-H}.

Finally, we can draw the following conclusions. The observed ^{15}N shieldings of amide nitrogens increase linearly with a decrease of the N–H bond length associated with the hydrogen bond; this can be justified by quantum chemical calculations. The ^{15}N shielding is applicable as a means for obtaining direct information about the nature of the hydrogen bond in the solid state, in addition to the ^{13}C shielding of the carbonyl carbons in the hydrogen bonds

REFERENCES

1 G A Webb and M Witanowski, Proc Indian Acad Sci (Chem Sci), 94 (1985) 241
2 G Harbison, J Herzfeld and R J Griffin, J Am Chem Soc, 103 (1981) 4752
3 T A Cross, J A Diverdi and S T, Opella, J Am Chem Soc, 104 (1982) 1759
4 T A Cross, M H Frey and S J Opella, J Am Chem Soc, 105 (1983) 7471
5 H G Forster, D Muller and H R Kricheldorf, Int J Biol Macromol, 5 (1983) 101
6 T H Huang, W W Achovchin, R G Griffin and C M Dobson, Biochemistry, 23 (1984) 5933
7 E O Stejskal, J Schaefer and R A McKay, J Magn Reson, 57 (1984) 471
8 C N Matthews, R Ludicky, J Schaefer, E O Stejskal and R A McKay, Origins Life, 14 (1984) 243
9 T A Cross and S T, Opella, J Mol Biol, 182 (1985) 367
10 B S Choi, J E Roberts, J N S Evans and M F Roberts, Biochemistry, 25 (1986) 557
11 J Schaefer, J R Garbow, G S Jacob, T M Forest and G E Wilson, Jr, Biochem Biophys Res Commun, 137 (1986) 736
12 P L Stewart, K G Valentine and S T Opella, J Magn Reson, 71 (1987) 45
13 (a) A Shoji, T Ozaki, T Fujito, K Deguchi and I Ando, Macromolecules, 20 (1987) 2441
(b) A Shoji, T Ozaki, T Fujito, K Deguchi, S Ando and I Ando, Macromolecules, 22 (1989) 2860
14 S Ando, I Ando, A Shoji and T Ozaki, J Am Chem Soc, 110 (1988) 3380
15 (a) L Zervas, D Borovas and E Gazis, J Am Chem Soc, 85 (1963) 3660
(b) E Wunsch, Synthese von Peptiden, Teil 1 and 2, Georg Thieme Verlag Stuttgart, 1974
(c) N Izumiya, T Kato, M Ohno and H Aoyagi, Peptide Synthesis, Maruzen, Tokyo, 1974
16 I Ando, H Saito, R Tabeta, A Shoji and T Ozaki, Macromolecules, 17 (1984) 457
17 R Ditchfield, D P Miller and J A Pople, J Chem Phys, 54 (1971) 4186
18 P D Ellis, G E Maciel and J M McIver, Jr, J Am Chem Soc, 94 (1972) 4069
19 I Ando and G A Webb, Theory of NMR Parameters, Academic Press, London, 1983
20 F A Momany, R F McGuire, J F Yan and H A Scheraga, J Phys Chem, 75 (1971) 2286
21 M Witanowski, L Stefaniak and G A Webb, Annual Reports on NMR Spectroscopy, Vol 18, Academic Press, London, 1986
22 H R Kricheldorf, Org Magn Reson, 15(2) (1981) 162

23 A B Biswas, E W Hughes, B D Sharma and J N Wilson, Acta Crystallogr , Sect B, 24 (1968) 40
24 T F Koetzle, W C Hamilton and R Parthasarathy, Acta Crystallogr , Sect B, 28 (1972) 2083
25 S N Rao and R Parthasarathy, Acta Crystallogr , Sect B, 29 (1973) 2379
26 V L Murali and E Subramanian, Int J Peptide Protein Res , 27 (1986) 472
27 V Lalita, E Subramanian and R Parthasarathy, Int J Peptide Protein Res , 27 (1986) 223
28 V Lalitha, E Subramanian and J Bordener, Indian J Pure Appl Phys , 23 (1985) 506
29 J P Glusker, H L Carrell, H M Berman, B Gallen and R M Peck, J Am Chem Soc , 99(2) (1977) 595
30 W M Carson and M L Hackert, Acta Crystallogr , Sect B, 34 (1978) 1275
31 Y Hiyama, C Niu, J V Silverton, A Bavoso and D A Torchia, J Am Chem Soc , 110 (1988) 2378
32 R Taylor, O Kennard and W Versichel, Acta Crystallogr , Sect B, 40 (1984) 280