Volume 281, number 1,2, 33–38 FEBS 09543 April 1991

ADONIS 001457939100251Y

Complete ^{1}H and ^{13}C assignment of Lys and Leu sidechains of staphylococcal nuclease using HCCH-COSY and HCCH-TOCSY 3D NMR spectroscopy

D.M. Baldisseri, J.G. Pelton, S.W. Sparks and D.A. Torchia

Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892, USA

Received 10 December 1990

Complete proton and carbon sidechain assignments are reported for 22 lysine and 11 leucine residues in staphylococcal nuclease, an enzyme with 149 residues. These assignments are readily obtained in a direct manner from the correlations observed in the 3D HCCH-COSY and HCCH-TOCSY spectra and the known protein backbone assignments. These assignments open the way to detailed studies of the sidechain structure and dynamics at the active site, in the hydrophobic core and on the surface of the protein.

Staphylococcal nuclease; Sidechain structure; Assignment; 3D NMR

1. INTRODUCTION

In the last few years substantial progress has been made in assigning the NMR signals of proteins having molecular weights in the 15–20 kDa range. One approach to obtain backbone assignments has been to apply a variety of double resonance two- and three-dimensional (2D and 3D) NMR experiments to proteins containing amino acids labeled with ^{2}H, ^{13}C and/or ^{15}N [1–5]. More recently it has been shown [6,7] that complete backbone assignments can be obtained more efficiently by applying sophisticated triple resonance 3D NMR techniques to proteins that are uniformly enriched with ^{15}N and ^{13}N. Once backbone assignments are made, recently developed 3D ^{1}H-^{13}C experiments [7–11] are an attractive way to extend the assignments to the sidechains. Herein we show that a combination of the HCCH-COSY [7,9,10] and HCCH-TOCSY [7,11] 3D experiments provide a particularly effective way to obtain assignments of sidechain protons and carbons of complex amino acids of staphylococcal nuclease (SNase) an enzyme containing 149 amino acids.

Sequential assignments of ca. 90% of the amide nitrogen, amide proton and α-proton signals of SNase have been reported [1–3,12]. Except for a few residues in the disordered N-terminus of the protein, the assignments of the α-protons have been completed, using ^{1}H-^{15}N NOESY-HMQC and HOHAHA-HMQC spectra [13,14] together with HCCH-COSY and HCCH-TOCSY spectra. In contrast with the nearly complete set of backbone assignments, only the proline sidechains [12], the aromatic sidechains [15] and some of the smaller sidechains [1–3] have been assigned. Perhaps the most difficult assignment problem involves the Lys residues because (i) the protein contains 23 Lys residues [16], and (ii) each residue contains a large number of protons whose signals resonate in a narrow spectral range. Assignment of the 11 Leu sidechains is also a challenge because, in addition to poor signal dispersion of the sidechain proton signals, the β-protons have small T_2 values because the Leu sidechains are normally buried and immobile. Assignment of these complex sidechains is important since it opens the way to study structure and dynamics at the protein active site, surface and hydrophobic core.

In our ititial attempt to assign these residues, we (i) obtained 2D HOHAHA spectra of deuterated proteins in which either the Lys or Leu residues were protonated, (ii) obtained ^{15}N edited NOESY spectra of proteins that were labeled with either [^{15}N]Lys or [^{15}N]Leu, and (iii) obtained ^{13}C edited NOESY spectra and ^{13}C edited

Correspondence address: D.A. Torchia, Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892, USA

Abbreviations: COSY, correlated spectroscopy; TOCSY, total related spectroscopy; HCCH-COSY, 3D ^{1}H-^{13}C-^{13}C-^{1}H correlation spectroscopy via $^{1}J_{CC}$ carbon couplings; HCCH-TOCSY, 3D ^{1}H-^{13}C-^{13}C-^{1}H total correlation spectroscopy with isotropic mixing of ^{13}C magnetization; NMR, nuclear magnetic resonance; NOESY, 2D nuclear Overhauser effect spectroscopy; HMQC, heteronuclear multiple quantum correlation spectroscopy; HOHAHA, homonuclear Hartmann-Hahn spectroscopy; DANTE, delays alternating with nutation for tailored excitation; DIPSI, decouping in the presence of scalar interactions

HMQC-RELAY spectra of SNase labeled with [Methyl-$^{13}C_2$]Leu. Although these experiments were partially successful, many proton assignments could not be made because of (i) signal overlap, (ii) the absence of proton J correlations, because J is often less than $1/T_2$, and (c) the difficulty of distinguishing sequential from long-range correlations in NOESY spectra. The assignment problem was solved by obtaining HCCH–COSY and HCCH-TOCSY spectra of a uniformly ^{13}C labeled sample of SNase.

2. MATERIALS AND METHODS

SNase enriched with ^{15}N and ^{13}C was obtained by growing *E. coli*, strain KL740 transformed with plasmid PNJS, on M9 media containing 99% NH_4Cl and 99% D-Glucose-$^{13}C_6$. The protein was prepared as described previously [1], and 14 mg of the wild-type Foggi [16] protein, residues 1–149, was obtained per gram of D-glucose. NMR spectra were recorded on a solution of [^{15}N, ^{13}C]Snase, 1.8 mM, in 99.996% D_2O buffer containing 100 mM NaCl, 5 mM pdTp, 10 mM $CaCl_2$ and 50 mM borate buffer, pD 6.8.

Spectra were recorded at 37°C on a Bruker AM-500 spectrometer that has been modified to eliminate overhead time at the end of every t_1 and t_2 increment. The 3D HCCH-COSY and HCCH-TOCSY experiments were performed using pulse sequences previously described [10,11]. In both experiments the ^1H carrier was positioned at 2.8 ppm and the ^{13}C carrier at 43 ppm. All proton pulses were applied with a 10.5 kHz rf field. ^{13}C pulses were applied with a 7.1 kHz (HCCH-COSY) and a 7.8 kHz (HCCH-TOCSY) rf field and the DANTE-type carbonyl inversion pulse was applied with a weak 1.4 kHz rf field. In the HCCH-TOCSY experiment, three loops of the DIPSI-3 sequence were used for isotropic mixing, corresponding to a duration of 24 ms, using a 2.9 kHz rf field. Acquisition times were 34, 12 and 51 ms in

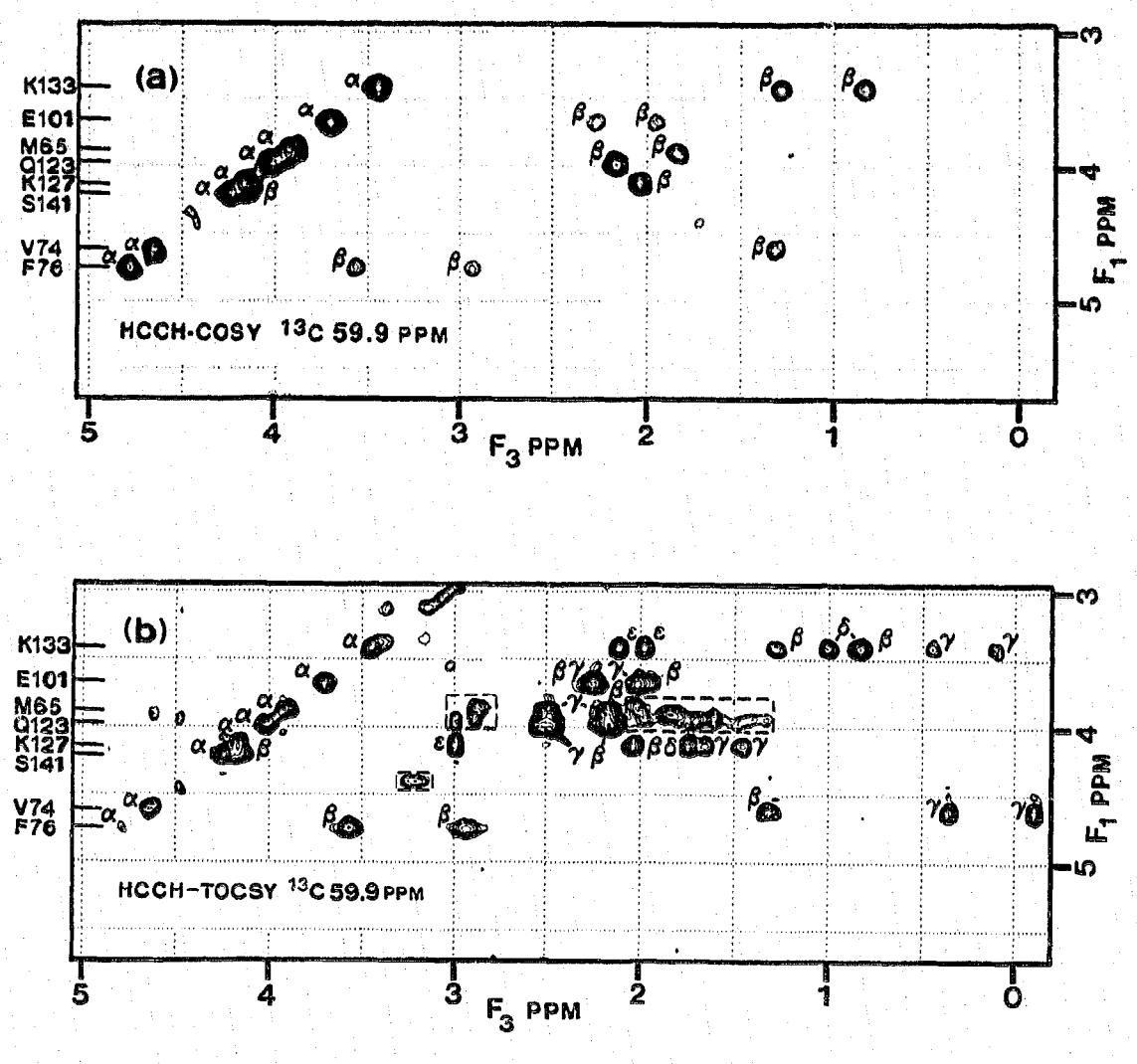

Fig. 1. Comparison of F1/F3 slices of (a) the 3D HCCH-COSY and (b) the 3D HCCH-TOCSY spectra of SNase. The F2 coordinate, 59.9 ppm, of each slice corresponds to the ^{13}C chemical shift of the carbons attached to the protons on the diagonal. The correlations within the dashed boxes arise from residues whose α-carbon signals are centered in adjacent slices.

the t_1, t_2 and t_3 dimensions, respectively. The size of the acquired 3D matrix was (128 complex) × (32 complex) × (512 real) and total measuring time was 60 h with a 0.7 s delay between scans. The 3D data sets were processed as described previously [9–11].

3. RESULTS AND DISCUSSION

The portion of the 3D HCCH-COSY spectrum of this sample (Fig. 1a) clearly shows the H^{α}-H^{β} correlations for 8 resisues in the protein whose α-carbons resonate at ca. 59.9 ppm. Although the HCCH-COSY experiment is analogous to the proton COSY experiment, the correlations in the HCCH-COSY spectrum are a consequence of large and uniform CH and CC one-bond J couplings [9,10]. They are therefore more intense and easier to interpret than the proton COSY correlations of SNase. For example, in Fig. 1a, when two H^{α}-H^{β} correlations are observed for a particular residue, the cross peaks have essentially equal intensity (K133); therefore a single strong H^{α}-H^{β} correlation is a clear signature of equivalent β-protons (K127).

In contrast, with the relatively simple HCCH-COSY spectrum, the HCCH-TOCSY spectrum (Fig. 1b) shows correlations from each α-proton to virtually all of the other aliphatic protons in the amino acid residue. In particular, correlations are evident from each α-proton to the corresponding protons in the long sidechains of Lys-127 and Lys-133. As is evident in Fig. 2, correlations involving the aliphatic protons of Lys-133 are seen in each of the 5 planes in which the five Lys-133 aliphatic carbons resonate. The observation of these correlations confirms the sidechain proton assignments indicated in Fig. 1, and, in addition, provides the assignments of all sidechain carbons. It is clear from Fig. 2 that the Lys-133 proton chemical shifts are significantly different from their expected random coil

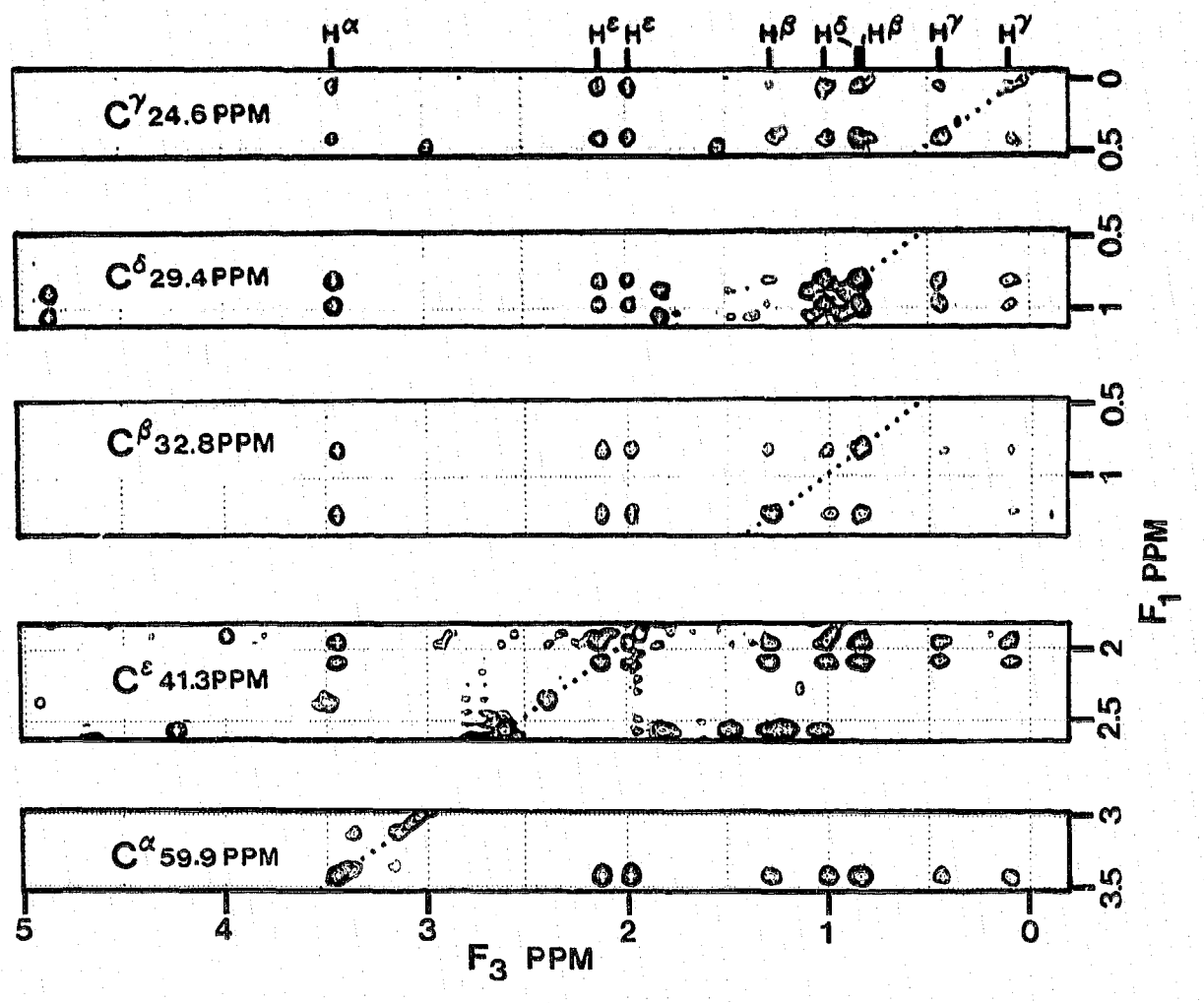

Fig. 2. A set of F1/F3 slices from the 3D HCCH-TOCSY spetrum of SNase showing the Lys-133 aliphatic proton correlations. The F2 coordinate of each slice corresponds to the chemical shift of the indicated aliphatic carbon. The proton assignments are indicated at the top of the figure, and the heavy dotted line defines the diagonal in each slice.

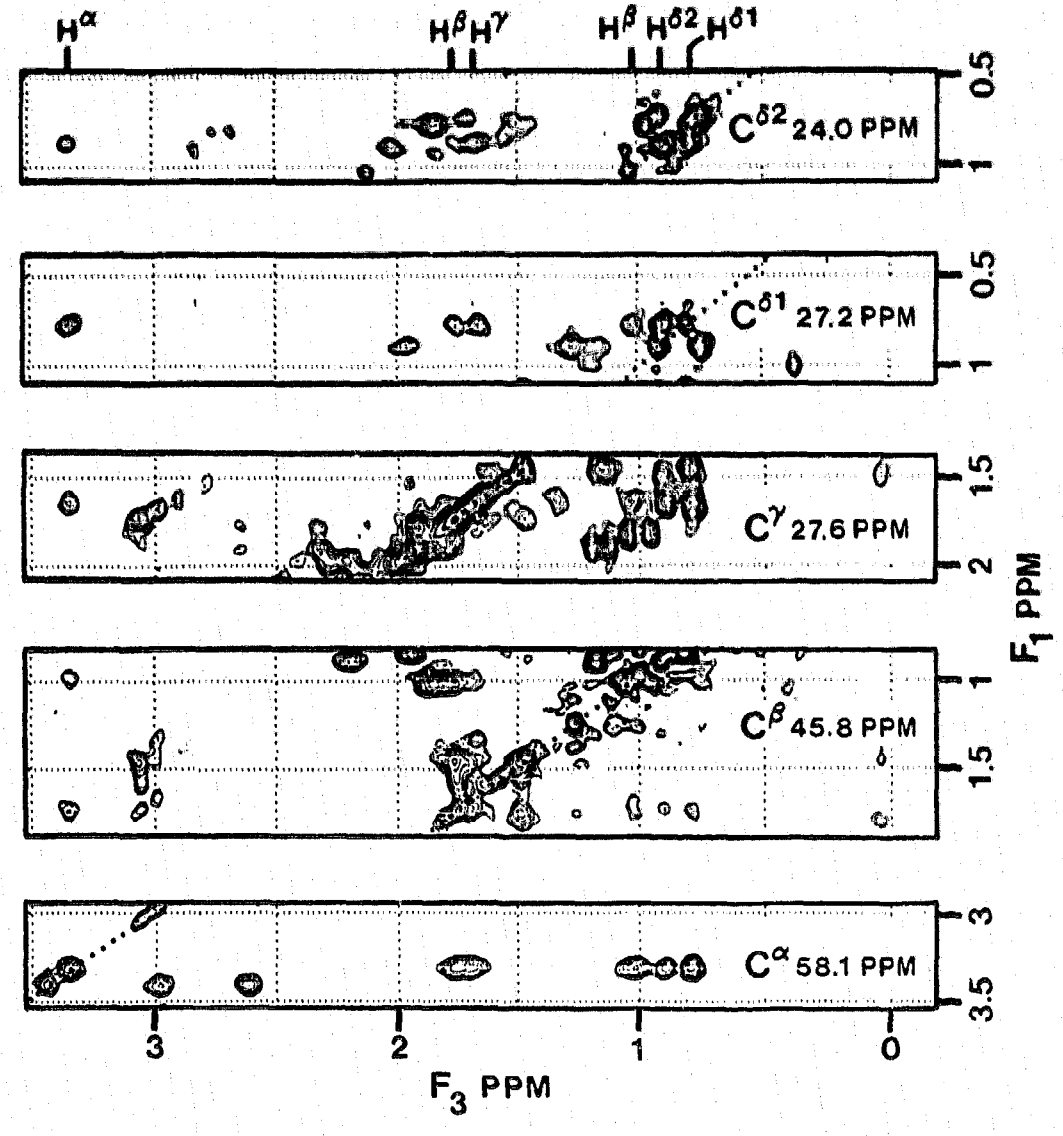

Fig. 3. A set of F1/F3 slices from the 3D HCCH-TOCSY spectrum of SNase showing the Leu-103 aliphatic correlations. The F2 coordinate of each slice corresponds to the ^{13}C chemical shift of the indicated aliphatic carbon. The proton assignments are indicated at the top of the figure, and the dotted lines define the diagonal in each slice.

values [17]. This is a consequence of the fact that many of the proton of the buried Lys-133 sidechain experience ring current shifts as a consequence of their proximity to the Trp-140 sidechain [18]. It is noteworthy that strong correlations are observed for Lys-133, an inflexible sidechain expected to have short T_2 values.

The HCCH-TOCSY spectrum (Fig. 3) of Leu-103, another residue having a large buried sidechain, exhibits correlations among all of its aliphatic protons. Systematic examination of the SNase HCCH-COSY and HCCH-TOCSY spectra, together with the known α-proton assignments, has the yielded assignments (Table I) of all aliphatic protons and carbons of all Lys and Leu residues except Lys-5. (Lys-5 and Lys-6 are in the disordered N-terminus of the protein and appear to have essentially identical aliphatic chemical shifts.)

The sidechain assignments open the way for studies of SNase solution structure using heteronuclear edited 3D NOESY spectroscopy [13,14,19,20] and SNase dynamics by means of ^{13}C relaxation measurements. Lys-84 is in the active site while Lys-45, -48 and -49 are in the functional omega loop at the active site. While most lysine sidechains are near the protein surface, a few lysine sidechains and all leucine sidechains are buried, with Leu-36, -37 and -38 located in the hydrophobic core of SNase. Therefore the assignments provided herein make it possible to probe the structure and dynamics at many important sites in SNase.

Table I

Chemical shifts[a] of $^1H^N$, $^{13}C^\alpha$ and $^{15}N^H$ nuclei of Lys[c] and Leu[c] residues in the SNase/pdTp/Ca^{2+} ternary complex.

Residue[d]	^{15}N	NH	C^α	H^α	C^β	$H^{\beta1}$	$H^{\beta2}$	C^γ	$H^{\gamma1}$	$H^{\gamma2}$	$C^{\delta1}$	$C^{\delta2}$	$H^{\delta1}$	$H^{\delta2}$	C^ε	$H^{\varepsilon1}$	$H^{\varepsilon2}$
K 5																	
K 6	122.0	8.11	56.2	4.39	33.7	1.75	1.86	24.7	1.45	1.51	28.9		1.75	1.75	42.2	3.05	3.05
K 9	124.1	8.40	56.1	4.87	35.1	1.42	1.53	25.8	0.80	1.35	29.2		0.90	1.05	40.8	1.80	1.80
K 16	114.6	8.04	56.7	4.44	35.9	1.84	1.84	24.2	1.30	1.30	29.1		1.66	1.68	42.0	2.96	2.96
K 24	127.5	9.41	56.3	5.43	35.0	1.69	2.04	25.7	1.36	1.36	30.0		1.63	1.63	41.9	2.83	2.83
K 28	127.7	9.28	60.4	3.59	33.6	1.76	1.82	24.5	1.16	1.27	30.0		1.60	1.66	41.8	3.01	3.01
K 45	123.1	8.09	56.0	4.31	33.0	1.64	1.82	24.7	1.32	1.32	29.1		1.64	1.64	42.0	2.96	2.96
K 48	117.6	9.21	56.9	4.61	34.9	2.02	1.90	25.0	1.47	1.58	28.8		1.75	1.75	41.9	3.03	3.03
K 49	119.6	8.91	57.1	4.25	33.7	1.23	1.78	25.4	1.03	1.28	28.9		1.26	1.47	41.4	2.57	2.57
K 53	125.7	8.53	59.0	3.80	32.1	1.66	1.55	25.0	0.85	1.11	29.1		1.53	1.53	41.9	2.76	2.76
K 63	120.2	8.09	57.5	3.59	30.1	1.75	1.38	25.0	0.49	0.83	29.4		1.51	1.51	42.1	2.97	2.97
K 64	117.3	7.90	59.4	3.94	32.2	1.75	1.75	24.9	1.31	1.39	29.3		1.58	1.58	42.0	2.86	2.86
K 70	125.8	10.12	57.8	4.36	32.5	1.94	2.04	25.2	1.56	1.63	28.8		1.73	1.73	42.2	3.04	3.04
K 71	121.4	8.86	55.6	4.65	35.7	1.70	1.86	24.9	1.24	1.49	29.2		1.66	1.66	42.0	2.90	2.90
K 78	119.4	10.22	58.0	4.23	33.7	1.85	1.98	25.0	1.62	1.67	29.3		1.83	1.83	42.2	3.12	3.12
K 84	116.1	7.81	57.8	4.08	31.4	1.56	1.69	23.2	0.07	0.83	29.1		1.53	1.43	42.1	2.65	2.74
K 97	121.4	7.86	54.5	4.68	33.4	1.76	1.88	24.7	1.43	1.49	28.8		1.74	1.74	42.1	3.06	3.06
K 110	117.4	7.52	54.0	5.10	35.5	1.82	1.89	24.8	1.21	1.32	29.6		1.60	1.60	41.8	2.52	2.63
K 116	125.4	8.70	56.3	4.02	32.1	1.78	1.78	24.9	1.33	1.52	29.5		1.71	1.71	42.0	3.01	3.01
K 127	122.4	7.96	60.1	4.13	32.0	2.02	2.02	25.1	1.43	1.64	29.3		1.71	1.71	42.0	2.98	2.98
K 133	117.3	8.13	59.7	3.44	32.5	1.26	0.81	24.4	0.43	0.08	29.3		0.84	0.99	41.2	1.95	2.09
K 134	122.4	7.75	59.3	3.97	32.5	1.95	1.95	24.8	1.46	1.50	29.2		1.67	1.67	42.0	2.97	2.97
K 136	116.8	7.79	56.9	3.56	29.8	1.82	2.07	25.0	1.40	1.42	29.3		1.73	1.78	42.3	3.07	3.07
L 7	122.2	8.26	54.9	4.56	41.8	1.77	1.77	28.1	1.80		24.9	22.1	0.96	1.04			
L 14	125.1	9.13	56.9	4.14	43.1	1.15	1.94	27.0	1.28		23.3	27.0	0.76	0.91			
L 25	127.3	9.34	53.3	5.20	45.8	1.78	1.44	27.0	1.48		25.4	24.8	0.02	0.75			
L 36	124.9	8.32	56.4	4.36	42.5	1.51	1.65	28.8	1.70		24.4	26.2	0.76	0.78			
L 37	122.0	7.43	56.3	3.95	45.9	0.75	1.08	27.8	1.28		26.6	26.5	0.41	1.19			
L 38	111.7	9.35	56.4	4.33	40.8	1.92	2.29	27.9	1.92		28.3	25.1	1.13	1.19			
L 89	126.1	8.25	52.9	5.22	43.3	1.70	1.13	25.9	1.48		25.2	22.2	0.67	0.68			
L 103	115.6	7.92	57.9	3.32	45.9	1.02	1.72	27.3	1.64		27.0	24.0	0.79	0.90			
L 108	115.4	7.77	53.7	4.29	44.0	1.22	1.43	26.5	1.22		25.8	22.7	0.48	0.74			
L 125	118.3	8.04	58.8	3.95	41.5	1.44	1.92	28.8	1.86		23.8	26.4	0.79	0.97			
L 137	117.1	7.55	55.2	4.18	44.5	1.37	1.53	26.9	1.69		23.0	25.5	0.86	1.02			

[a]In ppm.
[b]Relative to the water signal at 4.64 ppm in ^2H_2O and at 4.66 ppm in H_2O at 37°C, uncertainty 0.02 ppm.
[c]Relative to hypothetical internal (trimethylsilyl propionic acid.
[d]Relative to liquid NH_3.
[e]The superscripts 1 and 2 serve to distinguish methylene protons and are not meant to imply stereospecific assignments.

Acknowledgements: This work was supported by the Intramural AIDS Antiviral Program of the Office of the Director of the National Institutes of Health. We thank Drs Ad Bax, Mitsu Ikura and Lewis Kay for helpful discussions regarding the 3D pulse sequences and data processing. We are grateful to Professors John Gerlt and David Shortle for plasmids and transformed *E. coli*. We thank Rolf Tschudin for expert instrument support.

REFERENCES

[1] Torchia, D.A., Sparks, S.W. and Bax, A. (1989) Biochemistry 28, 5509–5524.
[2] Wang, J., LeMaster, D.M. and Markley, J.L. (1990) Biochemistry 29, 88–101.
[3] Wang, J., Hinck, A.P., Loh, S.N. and Markley, J.L. (1990) Biochemistry 29, 102–113.
[4] McIntosh, L.P., Wand, A.J., Lowry, D.F., Redfield, A.G. and Dahlquist, F.W. (1990) Biochemistry 29, 6341–6362.
[5] Driscoll, P.C., Clore, G.M., Marion, D., Wingfield, P.T. and Gronenborn, A.M. (1990) Biochemistry 29, 3542–3556.
[6] Ikura, M., Kay, L.E. and Bax, A. (1990) Biochemistry 29, 4659–4667.
[7] Clore, G.M., Bax, A., Driscoll, P.C., Wingfield, P.T. and Gronenborn, A.M. (1990) Biochemistry 29, 8172–8184.
[8] Fesik, S.W., Eaton, H.L., Olejniczak, E.T., McIntosh, L.P. and Dahlquist, F.W. (1990) J. Am. Chem. Soc. 112, 886–888.
[9] Kay, L.E., Ikura, M. and Bax, A. (1990) J. Am. Chem. Soc. 112, 888–889.
[10] Bax, A., Clore, G.M., Driscoll, P.C., Gronenborn, A.M., Ikura, M. and Kay, L.E. (1990) J. Magn. Reson. 87, 620–627.
[11] Bax, A., Clore, G.M. and Gronenborn, A.M. (1990) J. Magn. Reson. 88, 425–431.
[12] Torchia, D., Sparks, S.W., Young, P.E. and Bax, A. (1989) J. Am. Chem. Soc. 111, 8315–8317.
[13] Marion, D., Kay, L.E., Sparks, S.W., Torchia, D.A. and Bax, A. (1989) J. Am. Chem. Soc. 111, 1515–1517.
[14] Marion, D., Driscoll, P.C., Kay, L.E., Wingfield, P.T., Bax, A, Gronenborn, A.M. and Clore, G.M. (1989) Biochemistry 28, 6150–6156.

[15] Wang, J., Hinck, A.P., Loh, S.N. and Markley, J.L. (1990) Biochemistry 29, 4242–4253.
[16] Tucker, P.W., Hazen, E.E. Jr. and Cotton, F.A. (1979) Cell. Biochem. 23, 3–17.
[17] Wuthrich, K. (1986) NMR of Proteins and Nucleic Acids, p. 17, John Wiley & Sons, New York.
[18] Loll, P.J. and Lattman, E.E. (1989) Proteins: Structure, Function, and Genetics 5, 183–201.
[19] Ikura, M., Kay, L.E. and Bax, A. (1990) J. Magn. Reson. 86, 204–209.
[20] Zuiderweg, E.R.P., McIntosh, L.P., Dahlquist, F.W. and Fesik, S.W. (1990) J. Magn. Reson. 86, 210–216.