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Secondary Structure and Side-Chain ^{1}H and ^{13}C Resonance Assignments of Calmodulin in Solution by Heteronuclear Multi-Dimensional NMR Spectroscopy†

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Supplementary Material

TABLE 1S: ^{1}H, ^{13}C, and ^{15}N resonance assignmments of the side-chain groups in calmodulin-Ca^{2+}_4 (1.5 mM) at pH 6.3 and 36°C, 6.1 mM $CaCl_2$, 0.1 M KCl.

FIGURE 1S: A selected slice at $^{13}C(F_2)$ = 45.2 ppm of the HCCH-COSY spectrum of uniformly [^{15}N,^{13}C]-labeled CaM, illustrating connectivities originating from the two CαH protons of Gly-25, Gly-40, and Gly-98.

FIGURE 2S: The ^{15}N-^{1}H HMQC-J spectrum of uniformly [^{15}N]-labeled CaM. An enlarged cross peak is shown for Ile-100.

FIGURE 3S: Selected slices at different ^{13}C (F_2) chemical shifts of the ^{13}C NOESY-HMQC spectrum with 100 ms mixing time of uniformly [^{15}N,^{13}C]-labeled CaM, illustrating the $d_{\alpha\alpha}(i,j)$ connectivities between Thr-26 and Asp-64 and between Phe-99 and Asn-137.

TABLE 1S: ^{1}H, ^{13}C, and ^{15}N Resonance Assignments of the Side-chain Groups in Calmodulin-Ca^{2+}_4 (1.5 mM) at pH 6.3 and 36°C, 6.1 mM $CaCl_2$, 0.1 M KCl [a]

A1	1H:	4.15	1.57				
	13C:	51.8	18.8				
D2	1H:	4.67	2.72,2.60				
	13C:	54.8	41.2				
Q3	1H:	4.42	2.12,2.00	2.38,2.38	7.37,6.71(CONH2)[f]		
	13C:	55.6	30.2	33.7	180.0		
	15N:				111.1		
L4	1H:	4.70	1.75,1.56	1.76	0.96	0.94	
	13C:	54.5	43.7	27.3	26.7	23.7	
T5	1H:	4.51	4.74	1.38			
	13C:	60.7	71.3	21.7			
E6	1H:	4.02	2.08,2.08	2.42,2.38			
	13C:	60.1	29.2	36.3			
E7	1H:	4.12	2.08,1.96	2.40,2.33			
	13C:	60.0	28.9	36.7			
Q8	1H:	3.93	2.41,1.73	2.40,2.33	7.26,6.69(CONH2)[f]		
	13C:	58.7	29.2	34.9	176.5		
	15N:				111.1		
I9	1H:	3.77	2.00	1.85,1.15	1.14	0.89	
	13C:	66.3	37.8	30.0	17.5	13.3	
A10	1H:	4.13	1.57				
	13C:	55.5	17.9				
E11	1H:	4.17	2.03,1.98	2.42,2.42			
	13C:	59.4	29.1	35.6			
F12	1H:	5.02	3.49,3.49		7.20(δ)	7.34(ε)	n.d.[d](ζ)
	13C:	59.7	37.8		130.3[b]	130.0[c]	n.d.
K13	1H:	4.03	1.97,1.97	1.28,1.13	1.40,1.28	2.62,2.62	
	13C:	60.2	32.0	25.4	28.8	41.6	
E14	1H:	4.20	2.32,2.22	2.42,2.42			
	13C:	59.3	29.3	35.9			
A15	1H:	4.28	1.98				
	13C:	55.3	18.1				
F16	1H:	3.30	3.22,2.95		6.63(δ)	7.04(ε)	n.d.(ζ)
	13C:	62.1	39.6		131.8	129.0[b]	n.d.
S17	1H:	4.18	4.06,4.06				
	13C:	61.5	63.5				
L18	1H:	4.04	1.75,1.62	1.53	0.86	0.75	
	13C:	57.2	41.6	26.7	24.3	24.1	

(continued)

F19	1H:	4.29	2.72,2.72		7.35(δ)	7.28(ε)	n.d.(ζ)
	13C:	59.4	40.5		130.0[c]	129.9[c]	
D20	1H:	4.53	2.44,1.57				
	13C:	52.5	38.9				
K21	1H:	4.01	1.92,1.92	1.58,1.51	1.72,1.72	3.03,3.03	
	13C:	58.5	32.6	24.0	28.2	41.9	
D22	1H:	4.62	3.08,2.67				
	13C:	53.0	39.7				
G23	1H:	3.95					
	13C:	47.4					
D24	1H:	4.52	3.08,2.55				
	13C:	53.7	40.4				
G25	1H:	4.38,3.71					
	13C:	45.4					
T26	1H:	5.31	3.88	1.07			
	13C:	60.0	72.8	21.4			
I27	1H:	4.99	1.87	1.22	0.91	0.30	
	13C:	60.6	38.9		17.8	15.0	
T28	1H:	4.86	4.83	1.33			
	13C:	59.7	72.6	21.4			
T29	1H:	3.80	4.25	1.31			
	13C:	66.6	68.3	23.3			
K30	1H:	4.17	1.91,1.85	1.53,1.45	1.71,1.71	3.02,3.02	
	13C:	59.2	32.5	25.0	28.9	41.9	
E31	1H:	4.08	2.38,2.38[e]	2.35,2.35[e]			
	13C:	59.3	28.8	36.0			
L32	1H:	4.13	1.90,1.55	1.63	0.92	0.92	
	13C:	58.3	42.6	26.7	25.9	24.4	
G33	1H:	4.01,3.62					
	13C:	48.3					
T34	1H:	3.99	4.36	1.32			
	13C:	66.8	68.9	21.5			
V35	1H:	3.52	2.03	0.72	0.45		
	13C:	66.1	31.3	22.8	20.4		
M36	1H:	4.15	2.05,1.88	2.71,2.65	2.02[g]		
	13C:	59.1	n.d.	33.0	17.4		
R37	1H:	4.81	1.91,1.85	1.86,1.86	3.32,3.18		
	13C:	59.1	29.2	28.9	43.5		
S38	1H:	4.49	4.17,4.08				
	13C:	61.7	63.1				

(continued)

L39	1H:	4.51	1.90,1.85	1.85	0.87	0.87
	13C:	54.6	41.9	26.2	25.5	22.6
G40	1H:	4.24,3.81				
	13C:	45.5				
Q41	1H:	4.51	2.18,1.68	2.25,2.25	7.46,6.75 (CONH2)[f]	
	13C:	54.5	30.4	33.7	180.7	
	15N:				112.1	
N42	1H:	5.21	2.83,2.54		7.45,6.75 (CONH2)[f]	
	13C:	51.4	39.2		178.2	
	15N:				111.84	
P43	1H:	4.79	2.27,1.97	1.96,1.96	3.36,3.62	
	13C:	62.6	32.4	27.5	50.0	
T44	1H:	4.50	4.72	1.40		
	13C:	60.7	71.3	21.7		
E45	1H:	4.00	2.09,2.09	2.41,2.37		
	13C:	60.1	28.9	36.3		
A46	1H:	4.12	1.44			
	13C:	54.8	18.2			
E47	1H:	4.08	2.34,1.90[e]	2.38,2.34[e]		
	13C:	59.4	29.8	37.4		
L48	1H:	4.12	2.09,1.35	1.82	0.90	0.86
	13C:	58.0	42.6	27.1	25.7	24.3
Q49	1H:	3.89	2.22,2.19	2.48,2.48	7.47,6.80 (CONH2)[f]	
	13C:	58.6	28.1	34.1	180.3	
	15N:				112.5	
D50	1H:	4.48	2.83,2.71			
	13C:	57.6	40.3			
M51	1H:	4.06	2.32,2.10	2.80,2.54	2.09[g]	
	13C:	59.4	33.3	32.2	17.1	
I52	1H:	3.55	1.98	1.71,1.06	0.75	0.78
	13C:	64.9	37.4	29.1	16.1	
N53	1H:	4.42	3.02,2.90		7.75,6.85 (CONH2)[f]	
	13C:	56.0	38.0		176.5	
	15N:				111.5	
E54	1H:	4.08	2.15,2.04	2.45,2.32		
	13C:	59.0	30.3	36.2		
V55	1H:	4.47	2.41	0.95	0.86	
	13C:	61.0	32.7	19.3	21.8	
D56	1H:	4.67	2.76,2.56[e]			
	13C:	54.1	41.2			
A57	1H:	4.25	1.56			
	13C:	54.2	19.4			

(continued)

D58	1H:	4.61	3.05,2.69				
	13C:	53.0	39.7				
G59	1H:	3.95,3.85					
	13C:	47.1					
N60	1H:	4.67	3.35,2.68		7.65,6.99(CONH2)[f]		
	13C:	53.0	37.7		178.9		
	15N:				115.1		
G61	1H:	4.22,3.51					
	13C:	45.6					
T62	1H:	4.78	4.03	1.13			
	13C:	59.8	72.0	21.8			
I63	1H:	5.19	2.10	1.58,1.08	1.25	0.84	
	13C:	60.1	39.6	27.8	18.4	13.3	
D64	1H:	5.39	3.09,2.87				
	13C:	52.3	42.3				
F65	1H:	4.01	2.85,2.18		6.75(δ)	7.21(ε)	n.d.(ζ)
	13C:	63.7	n.d.		131.6	130.3[b]	n.d.
P66	1H:	3.95	2.23,1.94	2.23,1.94	3.81,3.76		
	13C:	66.8	31.1	28.5	49.3		
E67	1H:	4.14	2.60,2.13[e]	2.89, 2.53[e]			
	13C:	58.7	29.4	36.9			
F68	1H:	4.00	3.53,3.22		6.91(δ)	7.26(ε)	n.d.(ζ)
	13C:	61.3	40.0		132.0[b]	129.9[c]	n.d.
L69	1H:	3.39	1.57,1.21	1.07	0.66	0.63	
	13C:	58.0	41.0	25.7	25.5	24.0	
T70	1H:	3.81	4.35	1.24			
	13C:	66.5	68.4	21.8			
M71	1H:	3.81	2.12,2.00	2.45,2.17	1.82[g,e]		
	13C:	59.0	33.6	31.5	17.3		
M72	1H:	4.05	1.13,1.13[e]	1.38,1.38[e]	1.71[g,e]		
	13C:	55.9	31.0	32.2	17.8		
A73	1H:	4.17	1.38				
	13C:	53.7	18.4				
R74	1H:	4.13	1.91,1.88	1.81,1.68	3.13,3.13		
	13C:	58.1	30.4	27.3	43.6		
K75	1H:	4.26	1.95,1.85	1.51,1.51	1.68,1.68	2.95,2.95	
	13C:	57.1	32.5	25.0	28.8	41.8	
M76	1H:	4.42	2.21,2.12	2.73,2.65	2.13[g]		
	13C:	56.4	33.0	32.1	17.4		

(continued)

K77 1H:	4.37	1.93,1.85	1.49,1.49	1.72,1.72	3.02,3.02	
13C:	56.6	33.1	24.4	28.9	41.9	
D78 1H:	4.73	2.78,2.68				
13C:	54.7	41.2				
T79 1H:	4.36	4.30	1.25			
13C:	62.2	69.7	21.4			
D80 1H:	4.68	2.72,2.60				
13C:	54.8	41.5				
S81 1H:	4.49	4.03,3.98				
13C:	59.3	63.5				
E82 1H:	4.24	2.75,2.60[e]	2.37,2.37[e]			
13C:	58.6	29.4	36.3			
E83 1H:	4.15	2.09,1.98	2.42,2.36			
13C:	60.0	29.2	36.8			
E84 1H:	4.03	2.38,1.99	2.35,2.35[e]			
13C:	59.4	30.3	37.4			
I85 1H:	4.07	2.21	1.81,1.16	1.15	0.83	
13C:	64.6	37.5	29.1	18.8	13.3	
R86 1H:	4.20	2.08,1.90	1.75,1.58	3.02 3.02		
13C:	60.1	30.1	27.4	43.1		
E87 1H:	4.17	2.17,2.10	2.40,2.40[e]			
13C:	59.1	29.6	36.3			
A88 1H:	4.21	1.80				
13C:	55.2	17.7				
F89 1H:	3.23	3.20,3.03		6.63(δ)	7.02(ε)	7.05(ζ)
13C:	62.1	39.2		131.8	130.7[b]	n.d.
R90 1H:	3.94	2.00,2.00	1.95,1.74	3.25,3.25		
13C:	58.9	30.2	27.7	43.5		
V91 1H:	3.54	2.18	1.02	0.75		
13C:	65.7	31.5	22.8	20.8		
F92 1H:	4.28	2.71,2.71		7.35(δ)	7.28(ε)	7.24(ζ)
13C:	59.7	40.5		130.0[c]	129.9[c]	129.9[c]
D93 1H:	4.54	2.38,1.45				
13C:	52.6	38.3				
K94 1H:	3.97	1.90,1.90	1.55,1.50	1.69,1.69	2.91 2.91	
13C:	59.0	32.6	24.0	28.4	41.6	
D95 1H:	4.60	3.10,2.67				
13C:	53.0	39.7				
G96 1H:	3.86,3.82					
13C:	46.7					

(continued)

N97 1H:	4.68	3.44,2.72		7.99,7.29 (CONH2)		
13C:	52.9	38.1		179.8		
15N:				116.1		
G98 1H:	4.02,3.44					
13C:	45.2					
F99 1H:	5.19	2.66,2.62		6.95 (δ)	7.46 (ε)	7.37 (ζ)
13C:	55.9	44.1		132.0 [b]	131.0 [b]	130.0 [c]
I100 1H:	4.87	1.91	1.18,0.41	0.99	0.30	
13C:	60.4	38.6	27.0	18.0	14.8	
S101 1H:	4.90	4.47,4.02				
13C:	55.9	66.4				
A102 1H:	3.96	1.52				
13C:	56.1	18.0				
A103 1H:	4.07	1.47				
13C:	55.5	18.4				
E104 1H:	4.05	2.65,2.59 [e]	2.35 2.35 [e]			
13C:	59.4	29.7	36.3			
L105 1H:	4.16	1.90,1.56	1.69	0.92	0.86	
13C:	58.3	42.6	27.1	25.9	24.1	
R106 1H:	3.85	1.99,1.99	1.68,1.68	3.28,3.19		
13C:	59.9	30.4	27.8	43.7		
H107 1H:	4.38	3.38,3.32	8.08 (C2)	7.07 (C4)		
13C:	59.4	30.3	137.8	120.0		
V108 1H:	3.54	2.02	0.87	0.48		
13C:	66.1	31.7	22.9	20.7		
M109 1H:	4.37	2.20,2.05	2.74,2.65	2.08		
13C:	57.5	31.1	33.0	17.7		
T110 1H:	4.22	4.32	1.28			
13C:	65.6	68.7	21.3			
N111 1H:	4.54	2.84,2.84		7.37,6.54 (CONH2) [f]		
13C:	55.6	38.5		176.5		
15N:				111.1		
L112 1H:	4.37	1.89,1.75	1.82	0.92	0.86	
13C:	55.5	42.2	26.5	25.4	22.8	
G113 1H:	4.27,3.78					
13C:	45.4					
E114 1H:	4.48	2.00,1.79	2.28,2.18			
13C:	55.3	30.3	35.2			
K115 1H:	4.41	1.82,1.75	1.43,1.38	1.70,1.70	3.02,3.02	
13C:	55.7	32.1	24.4	28.9	41.9	

(continued)

L116	1H:	4.76	1.58,1.60	1.61	0.85	0.84
	13C:	54.1	44.8	27.4	26.5	23.8
T117	1H:	4.50	4.76	1.36		
	13C:	60.7	71.3	21.7		
D118	1H:	4.22	2.76,2.61			
	13C:	58.1	39.8			
E119	1H:	4.11	2.10,1.95	2.36,2.36[e]		
	13C:	59.4	29.2	36.2		
E120	1H:	4.19	2.20,2.09	2.43,2.36		
	13C:	59.3	30.0	36.3		
V121	1H:	3.66	2.25	1.07	1.02	
	13C:	67.1	31.4	22.4	23.7	
D122	1H:	4.38	2.80,2.67			
	13C:	57.8	40.5			
E123	1H:	4.03	2.13,2.07[e]	2.35,2.35		
	13C:	59.0	n.d.	35.9		
M124	1H:	4.06	2.30,2.07	2.75,2.59	1.93[g]	
	13C:	59.4	33.3	32.5	17.0	
I125	1H:	3.53	2.12	1.59,1.27	0.76	0.78
	13C:	64.1	36.5	28.5	16.3	11.5
R126	1H:	4.05	1.96,1.92	1.73,1.68	3.25,3.25	
	13C:	59.8	30.1	27.7	43.4	
E127	1H:	4.04	2.10,2.10	2.53,2.37		
	13C:	58.4	29.5	36.0		
A128	1H:	4.47	1.46			
	13C:	52.2	21.0			
N129	1H:	4.57	2.90,2.55			
	13C:	54.1	40.3			
I130	1H:	4.01	2.05	1.73,1.39	0.99	0.97
	13C:	63.2	38.7	28.3	17.6	12.7
D131	1H:	4.59	3.10,2.63			
	13C:	53.7	39.7			
G132	1H:	4.01,3.85				
	13C:	47.4				
D133	1H:	4.50	2.94,2.50			
	13C:	53.6	40.2			
G134	1H:	4.05,3.45				
	13C:	45.6				

(continued)

Q135	1H:	4.88	2.00,1.72	1.96,1.96	6.35,5.75 (CONH2)[f]		
	13C:	53.1	32.2	32.9	179.0		
	15N:				108.5		
V136	1H:	5.24	2.36	1.31	0.95		
	13C:	61.8	33.6	22.0	22.4		
N137	1H:	5.28	3.35,3.30		7.19,6.71 (CONH2)[f]		
	13C:	51.2	37.9		175.5		
	15N:				108.4		
Y138	1H:	3.49	2.43,2.12		6.33 (δ)	6.54 (ε)	
	13C:	62.8	37.7		132.1	117.9	
E139	1H:	3.70	2.12,1.98	2.40,2.32			
	13C:	60.4	28.8	37.1			
E140	1H:	4.06	2.60,2.25[e]	2.38,2.38[e]			
	13C:	58.6	29.2	35.7			
F141	1H:	4.04	3.43,3.23		6.98 (δ)	7.18 (ε)	6.96 (ζ)
	13C:	61.6	39.9		129.2	129.9	131.4
V142	1H:	3.21	1.94	0.80	0.59		
	13C:	67.3	31.4	21.3	22.8		
T143	1H:	3.78	4.28	1.24			
	13C:	66.6	68.3	21.8			
M144	1H:	4.13	2.12,2.12	2.57,2.37	2.08[g,e]		
	13C:	58.7	33.3	31.4	17.5		
M145	1H:	4.36	1.83,1.71	1.71,1.71	1.85[g,e]		
	13C:	55.6	32.2	n.d.	16.9		
T146	1H:	4.45	4.39	1.22			
	13C:	62.6	70.3	21.1			
S147	1H:	4.51	3.94,3.94				
	13C:	59.0	63.9				
K148	1H:	4.21	1.85,1.74	1.42,1.42	1.68,1.68	3.00,3.00	
	13C:	57.6	33.6	24.6	28.9	42.0	

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a ^{1}H and ^{13}C chemical shifts are reported relative to 3-(trimethylsilyl)propionic-d_4 acid and ^{15}N shifts relative to hypothetical internal liquid NH_3. The error in ^{1}H chemical shift is ±0.01 ppm, the error in ^{13}C shift ±0.2 ppm, and the error in ^{15}N shift ±0.15 ppm unless noted.

b The error in ^{13}C chemical shift is ±0.4 ppm due to spectral overlap.

c The error in ^{13}C chemical shift is ±0.8 ppm due to spectral overlap.

d Not determined.

(continued)

[e] Tentative assignment.

[f] The ^{1}H, ^{13}C, and ^{15}N assignments for the $CONH_2$ group of Gln and Asn residues are based on the NOE data obtained from the 100-ms ^{15}N NOESY-HMQC experiment and on the heteronuclear J connectivities obtained from the triple-resonance HNCO and HCACO experiments. The assignments of the $CONH_2$ groups of Gln-8 and Gln-41 are interchangeable.

[g] The ^{1}H and ^{13}C assignments for the Met methyl groups are based on the NOE data obtained from the 100-ms ^{13}C NOESY-HMQC experiment.

K-9228-M11

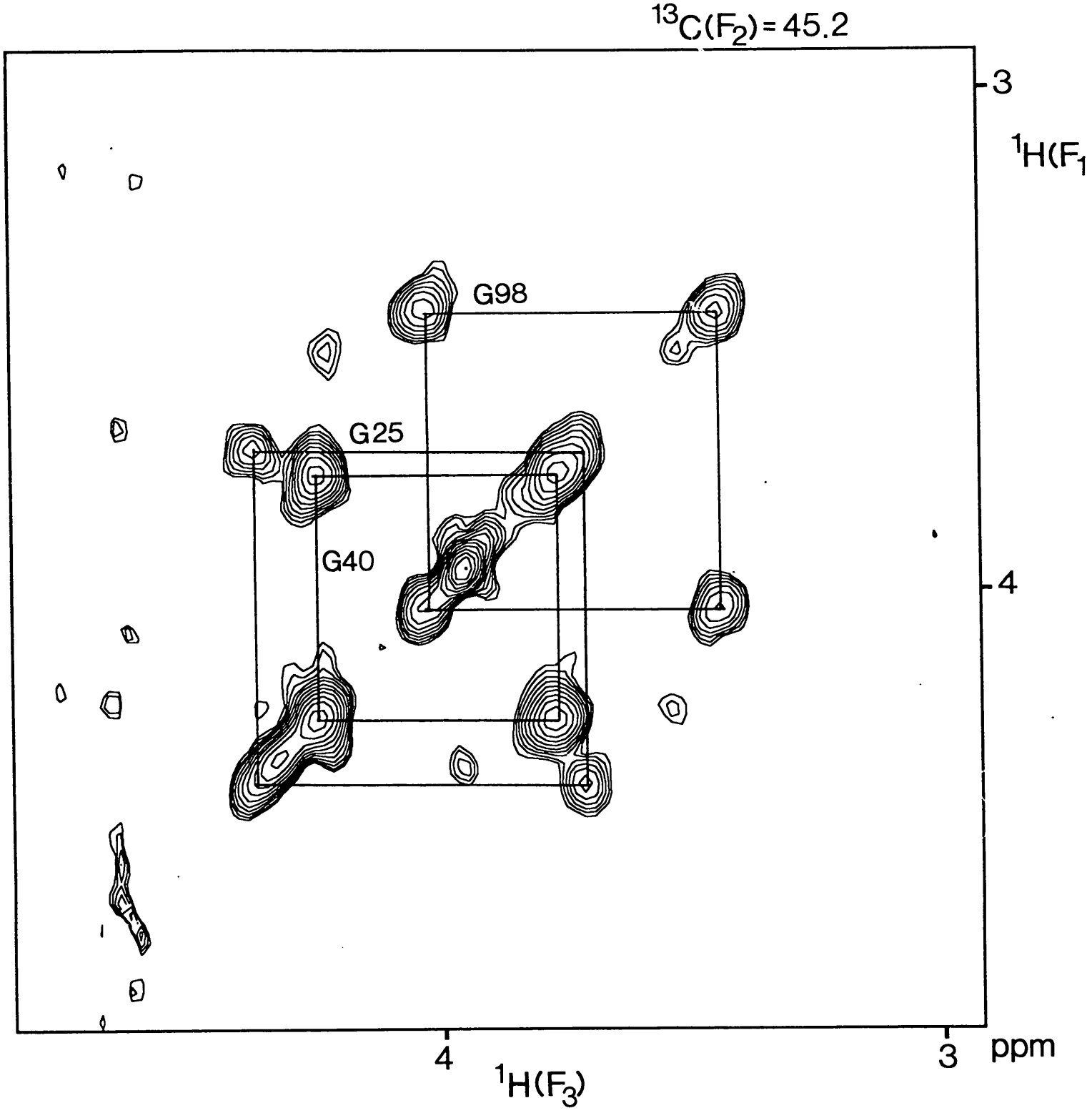

FIGURE 1S

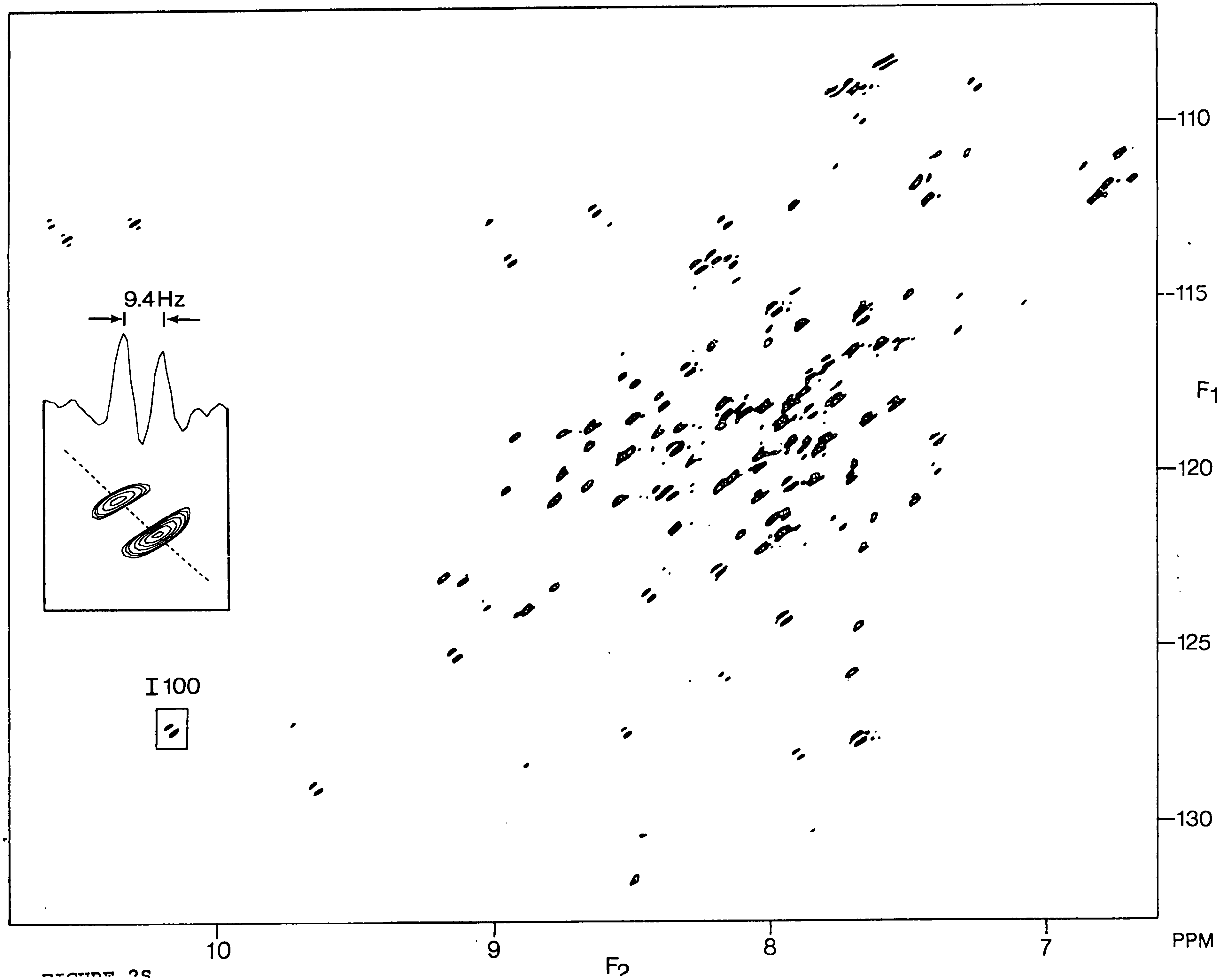

FIGURE 2S

K-9228-1M12

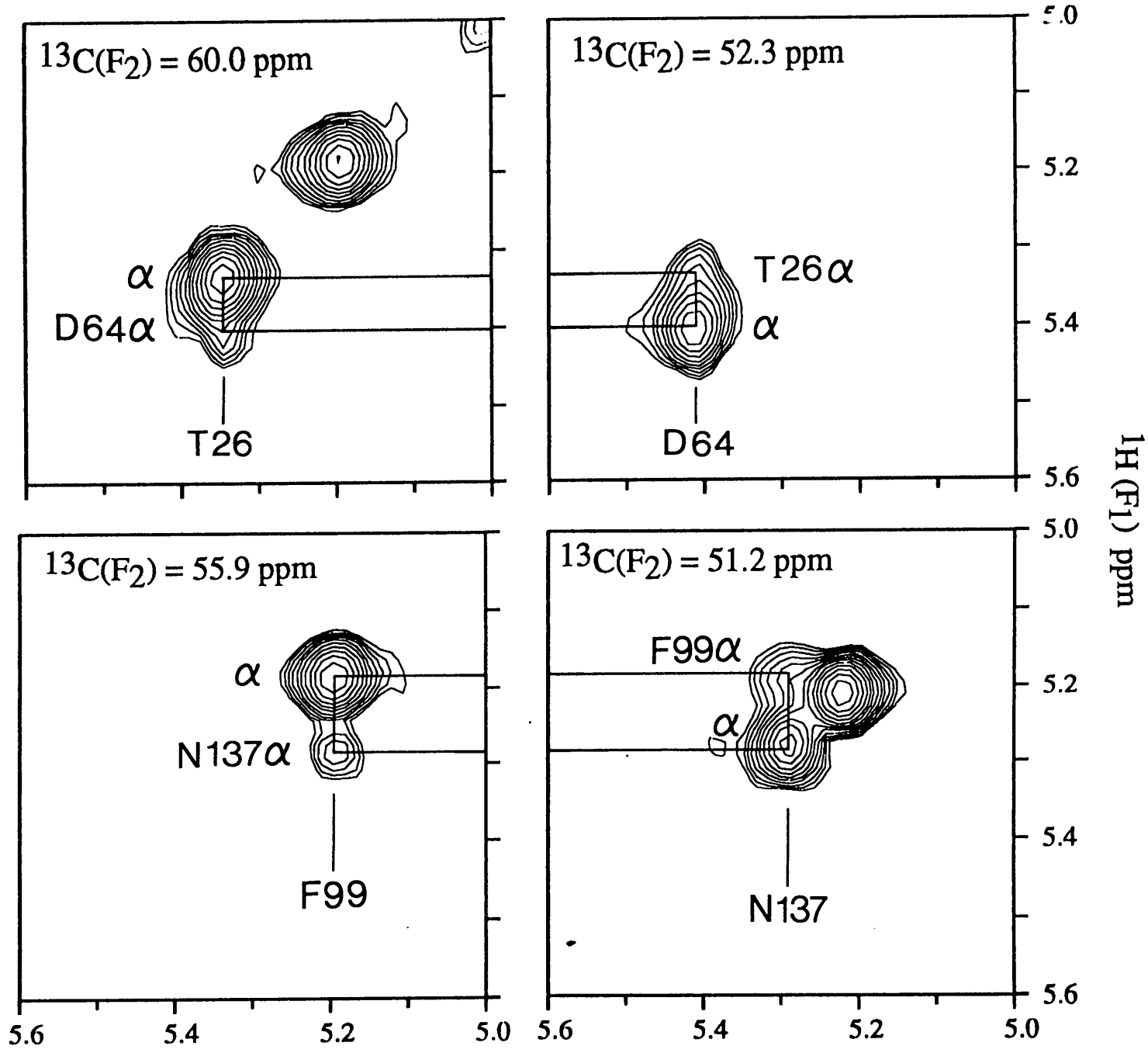

FIGURE 3S

4-9828-NMR