

Supporting Information

Studying ‘Invisible’ Excited Protein States in Slow Exchange with a Major State Conformation

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Table 1A. Values of $\Delta\omega$, R_2^G , R_2^E , R_1^G obtained from fits of CEST profiles recorded on the Abp1p-Ark1p system, 1°C, 11.7T to a global two-site exchange processes. The six residues that showed clear evidence for a second state (well resolved dips for both ground and excited states in profiles) were used in the fit. Optimal values of $(k_{ex}, p_E) = (130 \pm 4 \text{ s}^{-1}, 2.38 \pm 0.04\%)$ were obtained.

Residue	$\Delta\omega$ (ppm)	R_2^G (s^{-1})	R_2^E (s^{-1})	R_1^G (s^{-1})
15	-1.98 ± 0.02	8.27 ± 0.13	13.4 ± 4.6	1.67 ± 0.01
31	2.06 ± 0.02	9.73 ± 0.14	6.4 ± 4.8	1.64 ± 0.01
32	-8.82 ± 0.01	7.71 ± 0.14	19.9 ± 5.9	1.70 ± 0.01
33	2.27 ± 0.02	8.11 ± 0.15	13.2 ± 5.7	1.97 ± 0.01
34	4.52 ± 0.01	8.07 ± 0.14	27.1 ± 5.3	1.70 ± 0.01
37	-1.94 ± 0.03	9.79 ± 0.26	0.0 ± 4.9	1.96 ± 0.01

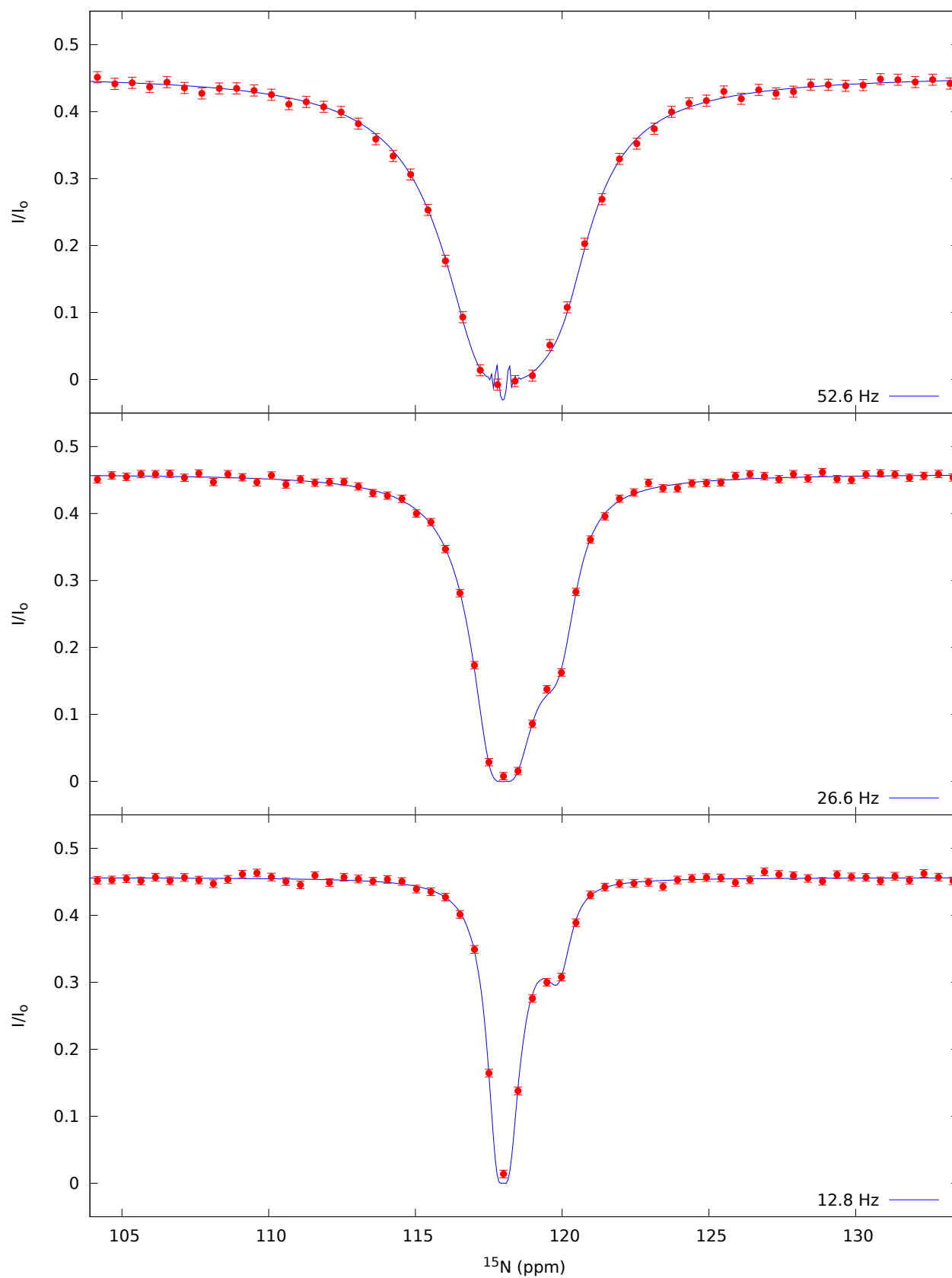
Table 1B. Values of $\Delta\omega$, R_2^G , R_2^E , R_1^G obtained from fits of all CEST profiles recorded on the Abp1p-Ark1p system, 1°C, 11.7T to a global two-site exchange processes. Values of $(k_{ex}p_E)$ were fixed to (130 s⁻¹, 2.38%) and profiles for all of the residues fit simultaneously.

Residue	$\Delta\omega$ (ppm)	R_2^G (s ⁻¹)	R_2^E (s ⁻¹)	R_1^G (s ⁻¹)
1	-0.16 ± 0.07	5.33 ± 0.25	0.7 ± 10.2	1.60 ± 0.01
3	0.26 ± 0.05	8.71 ± 0.36	4.8 ± 14.5	1.87 ± 0.01
4	0.37 ± 0.06	8.78 ± 0.39	2.6 ± 15.5	2.02 ± 0.01
5	0.47 ± 0.04	9.63 ± 0.30	16.4 ± 11.8	1.88 ± 0.01
6	0.11 ± 0.06	7.09 ± 0.45	94.7 ± 28.5	1.97 ± 0.01
7	-0.11 ± 0.21	9.28 ± 1.39	12.3 ± 60.4	1.98 ± 0.02
8	-0.76 ± 0.03	8.87 ± 0.25	5.7 ± 8.5	1.92 ± 0.01
9	0.36 ± 0.03	8.48 ± 0.23	0.1 ± 8.8	1.66 ± 0.01
10	0.57 ± 0.04	9.57 ± 0.24	19.4 ± 9.5	1.96 ± 0.01
11	-0.41 ± 0.03	8.52 ± 0.21	3.8 ± 8.7	1.75 ± 0.01
12	0.28 ± 0.06	8.46 ± 0.32	2.4 ± 13.4	1.81 ± 0.01
13	-0.49 ± 0.03	8.72 ± 0.25	10.9 ± 9.8	1.81 ± 0.01
14	-0.53 ± 0.03	8.05 ± 0.22	3.2 ± 8.1	1.64 ± 0.01
15	-1.98 ± 0.01	8.27 ± 0.10	13.4 ± 3.6	1.67 ± 0.01
16	-0.64 ± 0.02	8.18 ± 0.19	18.6 ± 7.7	1.79 ± 0.01
17	-1.07 ± 0.02	9.64 ± 0.14	7.1 ± 4.5	1.86 ± 0.01
18	-0.57 ± 0.05	9.49 ± 0.34	1.3 ± 12.2	2.05 ± 0.01
19	-0.39 ± 0.03	8.16 ± 0.19	4.8 ± 7.6	1.67 ± 0.01
20	0.21 ± 0.09	9.19 ± 0.65	17.1 ± 28.3	1.98 ± 0.01
21	-0.16 ± 0.09	9.45 ± 0.60	2.9 ± 24.9	1.86 ± 0.01
22	0.25 ± 0.06	7.92 ± 0.39	58.4 ± 21.5	1.78 ± 0.01
23	-0.26 ± 0.06	8.36 ± 0.35	15.2 ± 15.7	1.91 ± 0.01
24	0.27 ± 0.07	9.65 ± 0.38	7.4 ± 16.3	1.89 ± 0.01
25	0.25 ± 0.06	8.53 ± 0.37	2.6 ± 14.8	1.77 ± 0.01
27	-0.20 ± 0.09	8.39 ± 0.77	39.7 ± 38.3	1.99 ± 0.01
28	-0.14 ± 0.07	8.46 ± 0.56	47.8 ± 28.9	1.81 ± 0.01
29	-0.06 ± 0.23	9.12 ± 2.46	22.7 ± 110	1.98 ± 0.03
30	-0.54 ± 0.03	9.35 ± 0.23	3.0 ± 7.7	1.87 ± 0.01
31	2.06 ± 0.02	9.73 ± 0.13	6.3 ± 4.2	1.64 ± 0.01
32	-8.82 ± 0.01	7.71 ± 0.13	19.9 ± 5.2	1.70 ± 0.01
33	2.27 ± 0.02	8.12 ± 0.18	13.1 ± 6.9	1.97 ± 0.01
34	4.52 ± 0.01	8.07 ± 0.11	27.7 ± 4.4	1.70 ± 0.01
35	-1.02 ± 0.02	8.36 ± 0.16	22.2 ± 6.0	1.84 ± 0.01
36	-1.13 ± 0.03	8.47 ± 0.23	12.8 ± 8.0	1.83 ± 0.01
37	-1.95 ± 0.04	9.91 ± 0.28	4.0 ± 8.8	1.96 ± 0.01
38	-0.30 ± 0.07	9.29 ± 0.51	2.5 ± 19.4	1.90 ± 0.01
39	0.47 ± 0.05	9.60 ± 0.34	18.1 ± 13.8	1.93 ± 0.01
40	0.30 ± 0.03	2.94 ± 0.14	0.8 ± 5.5	1.48 ± 0.01
41	0.33 ± 0.06	9.51 ± 0.33	2.4 ± 13.3	2.00 ± 0.01
42	-0.35 ± 0.04	8.55 ± 0.25	0.2 ± 10.1	1.96 ± 0.01
43	-0.17 ± 0.13	8.43 ± 0.71	18.4 ± 33.0	1.89 ± 0.01
44	-0.37 ± 0.04	8.49 ± 0.29	1.5 ± 11.2	1.96 ± 0.01
45	-0.21 ± 0.08	8.98 ± 0.46	0.6 ± 18.4	1.92 ± 0.01
46	-0.29 ± 0.06	8.36 ± 0.44	25.1 ± 20.2	1.91 ± 0.01
47	-0.05 ± 0.04	7.20 ± 0.37	66.9 ± 20.6	1.70 ± 0.01
48	0.20 ± 0.10	8.93 ± 0.68	23.8 ± 31.6	1.91 ± 0.01
49	-0.83 ± 0.03	9.55 ± 0.22	15.9 ± 8.5	1.84 ± 0.01
50	0.19 ± 0.12	9.78 ± 0.69	1.2 ± 27.9	2.04 ± 0.01
52	-1.14 ± 0.03	12.14 ± 0.23	44.7 ± 9.1	1.88 ± 0.01
53	0.87 ± 0.04	12.74 ± 0.33	0.4 ± 10.1	1.85 ± 0.01

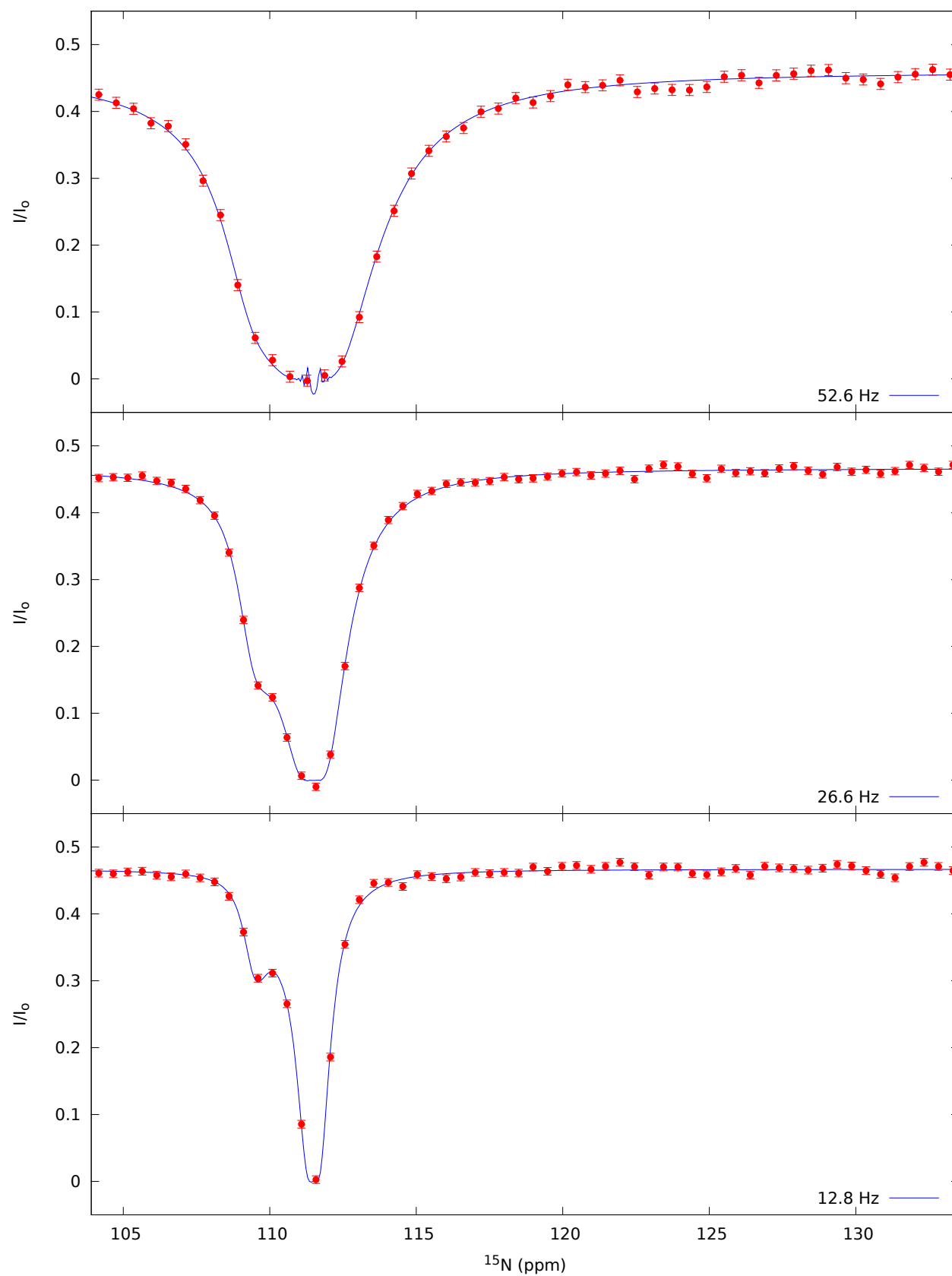
54	0.24 ± 0.09	9.45 ± 0.64	5.9 ± 26.8	1.99 ± 0.01
55	-0.52 ± 0.06	9.97 ± 0.32	176.8 ± 26.0	1.95 ± 0.01
56	0.33 ± 0.06	9.46 ± 0.42	35.4 ± 20.0	1.87 ± 0.01
57	-0.28 ± 0.06	9.26 ± 0.38	39.7 ± 19.0	1.76 ± 0.01
58	-0.25 ± 0.07	8.89 ± 0.46	2.2 ± 18.0	1.91 ± 0.01
59	-0.29 ± 0.06	6.60 ± 0.37	0.5 ± 14.9	1.71 ± 0.01

Supporting Figure 1. Intensity profiles (red) and best fits (blue) from the global fit of the six residues in the Abp1p-Ark1p exchanging system, 1°C, 11.7T, showing clear evidence of a second state.

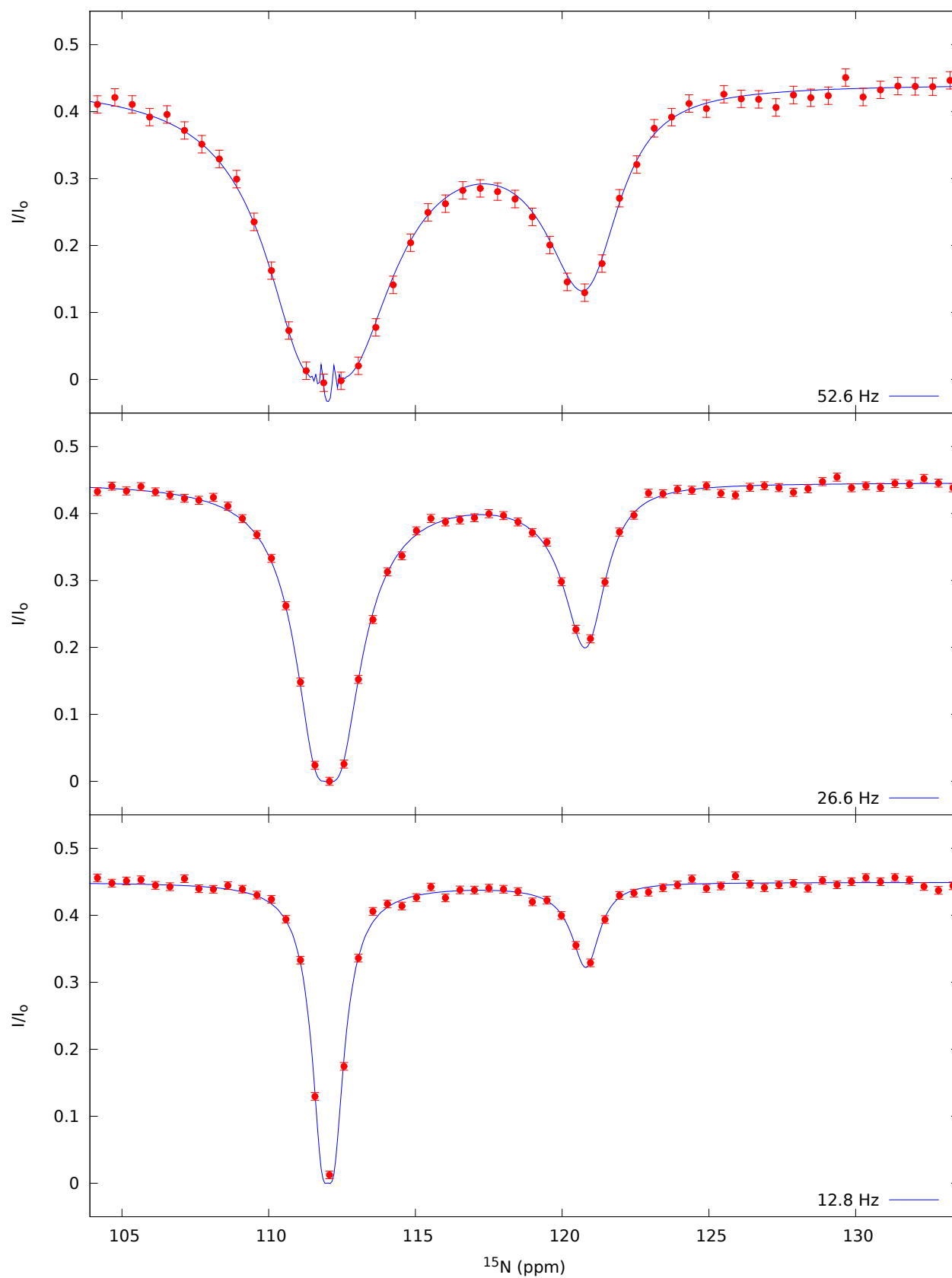
Residue 15



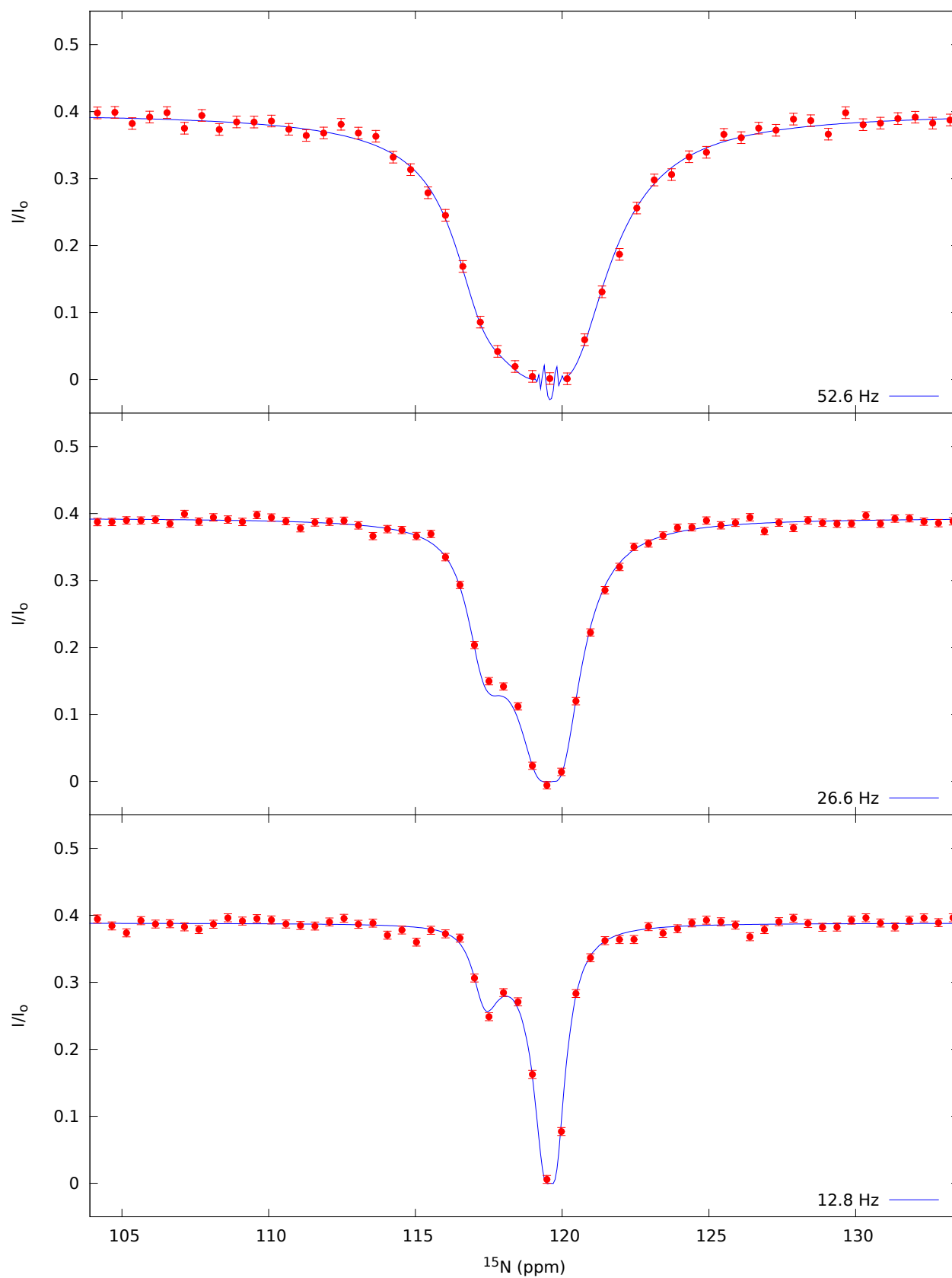
Residue 31



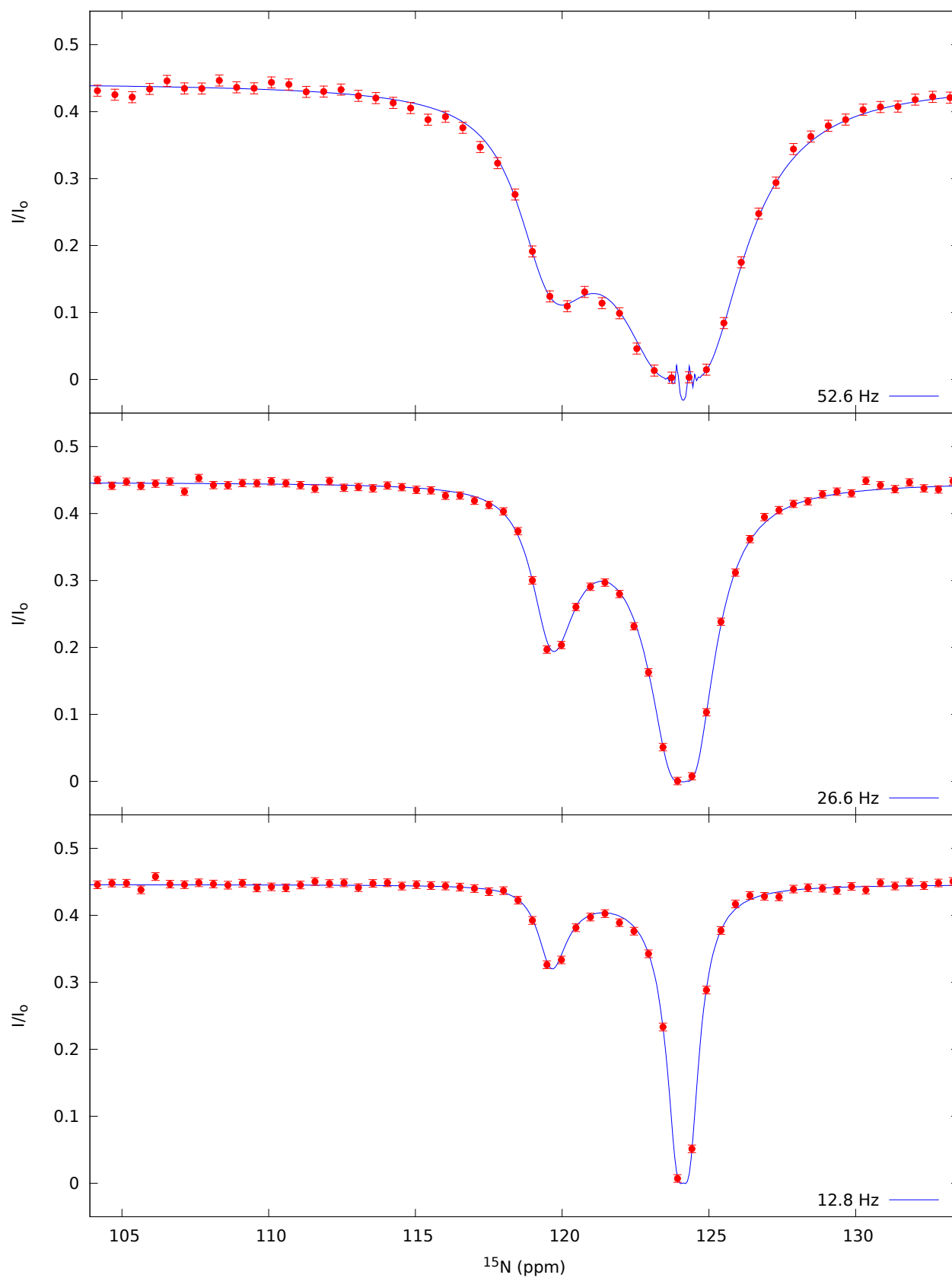
Residue 32



Residue 33



Residue 34



Residue 37

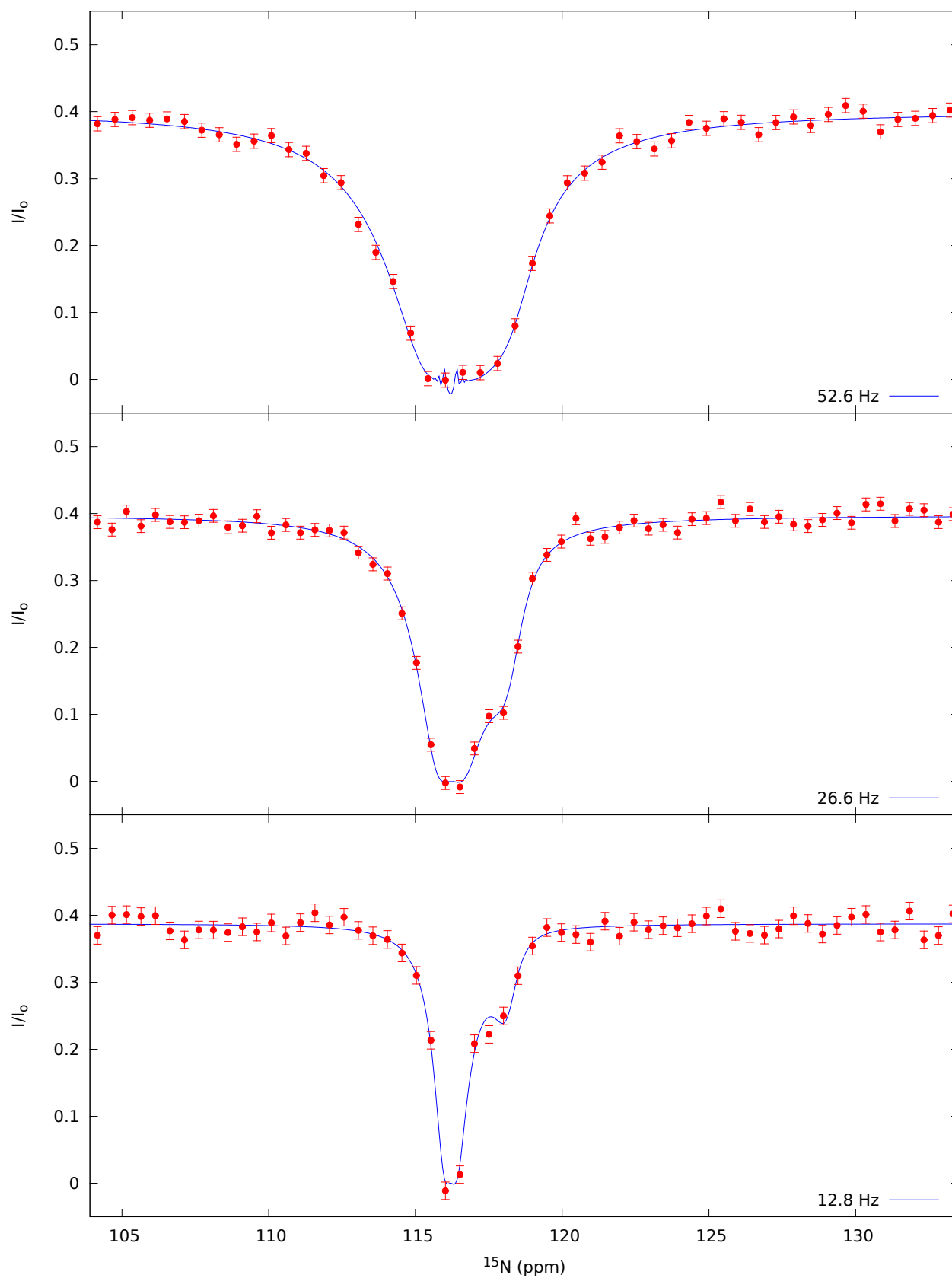


Table 2A. Values of $\Delta\omega$, R_2^G , R_2^E , R_1^G obtained from fits of CEST profiles recorded on the A39G FF domain 1°C, 11.7T to a global two-site exchange processes. The 37 residues that showed clear evidence for a second state (well resolved dips for both ground and excited states in profiles) were used in the fit. Optimal values of $(k_{ex}, p_E) = (51.6 \pm 0.7 \text{ s}^{-1}, 1.65 \pm 0.014\%)$ were obtained.

Residue	$\Delta\omega$ (ppm)	R_2^G (s^{-1})	R_2^E (s^{-1})	R_1^G (s^{-1})
10	1.68 ± 0.02	10.36 ± 0.11	26.6 ± 5.0	1.45 ± 0.01
13	4.23 ± 0.02	14.01 ± 0.09	119.4 ± 6.8	1.22 ± 0.01
15	1.89 ± 0.02	12.00 ± 0.08	35.2 ± 3.9	1.39 ± 0.01
18	2.15 ± 0.01	12.36 ± 0.08	38.7 ± 4.0	1.40 ± 0.01
22	3.34 ± 0.02	12.47 ± 0.08	74.7 ± 5.3	1.42 ± 0.01
25	2.76 ± 0.02	12.88 ± 0.09	62.2 ± 5.2	1.46 ± 0.01
26	5.25 ± 0.02	12.58 ± 0.08	92.1 ± 5.6	1.42 ± 0.01
27	-1.94 ± 0.02	12.77 ± 0.10	64.1 ± 6.3	1.43 ± 0.01
28	6.70 ± 0.02	11.95 ± 0.08	203.8 ± 9.5	1.44 ± 0.01
29	5.85 ± 0.02	12.27 ± 0.09	162.2 ± 9.4	1.41 ± 0.01
33	4.28 ± 0.02	11.89 ± 0.08	85.2 ± 5.6	1.48 ± 0.01
37	4.78 ± 0.02	12.89 ± 0.09	100.1 ± 6.7	1.38 ± 0.01
38	2.43 ± 0.01	13.06 ± 0.10	18.3 ± 5.0	1.41 ± 0.01
39	1.49 ± 0.01	12.77 ± 0.09	29.2 ± 4.3	1.43 ± 0.01
41	6.15 ± 0.02	14.00 ± 0.08	102.8 ± 5.9	1.36 ± 0.01
42	6.23 ± 0.02	12.74 ± 0.10	164.0 ± 9.9	1.41 ± 0.01
43	12.46 ± 0.03	12.76 ± 0.10	216.5 ± 12.0	1.41 ± 0.01
44	3.45 ± 0.03	13.62 ± 0.11	196.2 ± 12.1	1.38 ± 0.01
45	3.80 ± 0.02	13.21 ± 0.08	161.0 ± 8.4	1.36 ± 0.01
48	2.27 ± 0.01	12.11 ± 0.09	28.6 ± 3.6	1.46 ± 0.01
50	8.20 ± 0.01	12.22 ± 0.08	69.7 ± 4.5	1.43 ± 0.01
51	1.54 ± 0.02	13.12 ± 0.10	58.1 ± 6.4	1.48 ± 0.01
52	8.42 ± 0.01	14.23 ± 0.15	21.9 ± 6.1	1.41 ± 0.01
53	-2.80 ± 0.01	10.87 ± 0.07	47.1 ± 4.4	1.33 ± 0.01
54	3.25 ± 0.01	10.55 ± 0.07	69.0 ± 4.7	1.32 ± 0.01
55	-6.38 ± 0.02	11.88 ± 0.10	34.8 ± 4.3	1.45 ± 0.01
56	3.64 ± 0.02	12.17 ± 0.09	87.5 ± 6.3	1.41 ± 0.01
59	6.30 ± 0.01	12.81 ± 0.10	38.4 ± 5.3	1.43 ± 0.01
60	2.40 ± 0.01	12.68 ± 0.09	33.4 ± 4.5	1.38 ± 0.01
61	2.22 ± 0.01	12.96 ± 0.09	18.2 ± 3.5	1.44 ± 0.01
63	2.93 ± 0.01	12.37 ± 0.09	39.4 ± 4.6	1.43 ± 0.01
64	2.08 ± 0.01	12.54 ± 0.09	20.2 ± 3.3	1.41 ± 0.01
65	-1.64 ± 0.01	11.93 ± 0.09	20.8 ± 4.5	1.43 ± 0.01
66	4.18 ± 0.01	12.29 ± 0.08	48.9 ± 4.7	1.45 ± 0.01
67	8.04 ± 0.01	12.20 ± 0.08	61.9 ± 4.2	1.39 ± 0.01
68	4.38 ± 0.01	10.57 ± 0.07	43.6 ± 3.9	1.46 ± 0.01
69	1.85 ± 0.01	7.75 ± 0.07	11.7 ± 3.6	1.56 ± 0.01

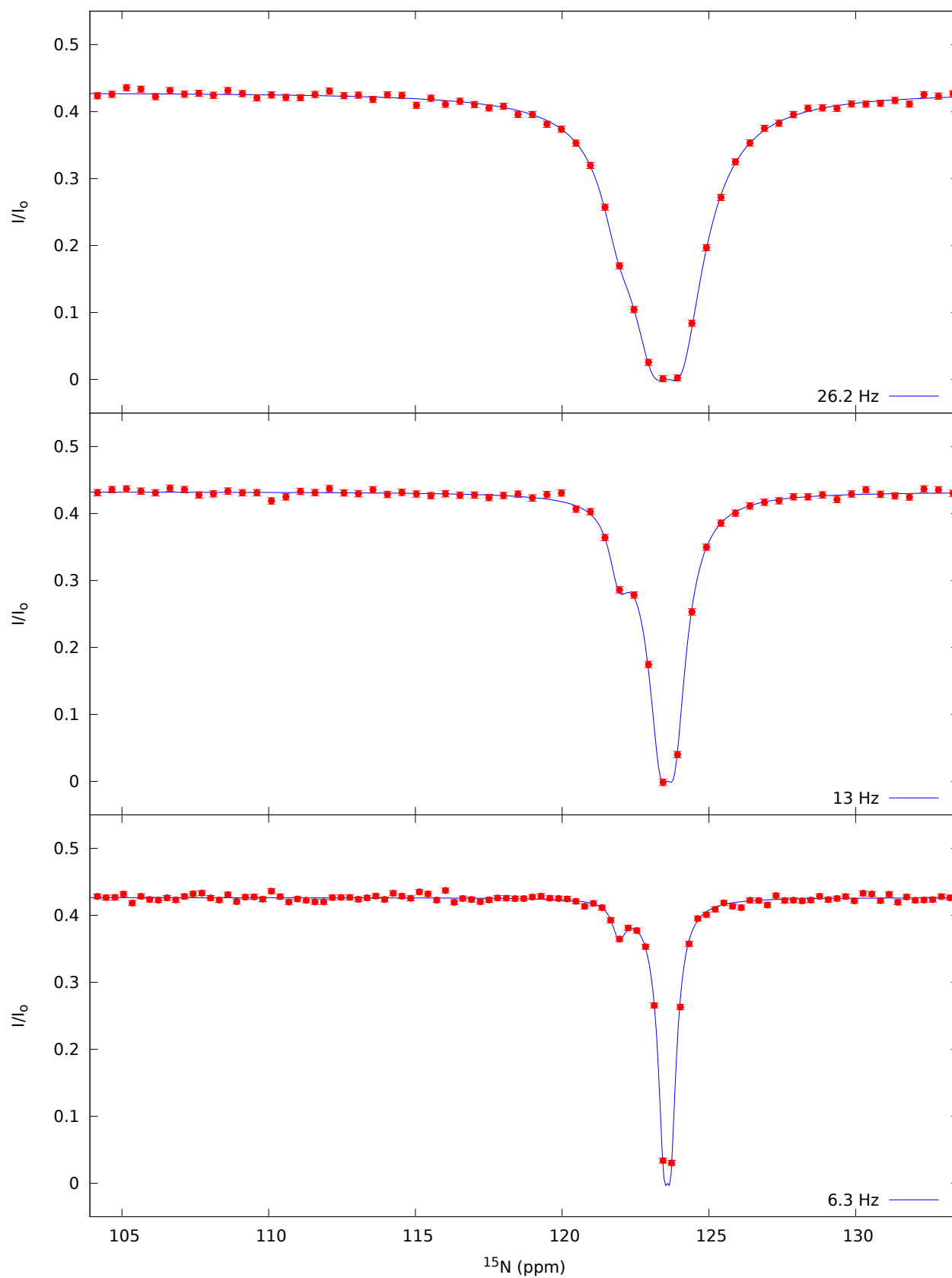
Table 2B. Values of $\Delta\omega$, R_2^G , R_2^E , R_1^G obtained from fits of all CEST profiles recorded on the A39G FF domain, 1°C, 11.7T to a global two-site exchange processes. Values of $(k_{ex}p_E)$ were fixed to (51.6 s⁻¹,1.65%) and profiles for all of the residues fit simultaneously.

Residue	$\Delta\omega$ (ppm)	R_2^G (s ⁻¹)	R_2^E (s ⁻¹)	R_1^G (s ⁻¹)
3	0.18 ± 0.03	1.81 ± 0.09	0.9 ± 5.2	1.19 ± 0.01
5	-0.13 ± 0.03	2.74 ± 0.08	0.3 ± 4.3	1.43 ± 0.01
6	-0.22 ± 0.01	3.57 ± 0.07	0.0 ± 3.3	1.49 ± 0.01
7	-0.13 ± 0.02	5.07 ± 0.06	0.5 ± 3.7	1.56 ± 0.01
8	-0.96 ± 0.01	8.33 ± 0.07	7.9 ± 2.9	1.54 ± 0.01
9	-0.22 ± 0.03	7.58 ± 0.11	19.1 ± 7.1	1.53 ± 0.01
10	1.68 ± 0.02	10.32 ± 0.11	27.2 ± 5.2	1.45 ± 0.01
11	1.36 ± 0.05	16.17 ± 0.33	42.5 ± 18.4	1.41 ± 0.01
12	0.45 ± 0.02	13.96 ± 0.14	1.0 ± 7.0	1.37 ± 0.01
13	4.23 ± 0.02	13.96 ± 0.08	120.2 ± 6.3	1.23 ± 0.01
14	-0.32 ± 0.03	11.67 ± 0.15	14.6 ± 8.8	1.45 ± 0.01
15	1.89 ± 0.01	11.95 ± 0.07	35.7 ± 3.4	1.39 ± 0.01
16	1.30 ± 0.01	11.16 ± 0.09	26.0 ± 4.8	1.44 ± 0.01
17	-1.11 ± 0.02	12.92 ± 0.11	13.1 ± 3.9	1.47 ± 0.01
18	2.15 ± 0.01	12.31 ± 0.09	39.3 ± 4.3	1.40 ± 0.01
19	0.92 ± 0.02	12.62 ± 0.08	39.1 ± 4.6	1.41 ± 0.01
20	-0.18 ± 0.07	12.79 ± 0.40	66.5 ± 33.9	1.48 ± 0.01
21	0.41 ± 0.03	11.45 ± 0.12	33.4 ± 7.8	1.52 ± 0.01
22	3.34 ± 0.02	12.42 ± 0.08	75.1 ± 5.4	1.42 ± 0.01
23	-0.17 ± 0.04	12.77 ± 0.25	33.9 ± 18.1	1.44 ± 0.01
24	-0.36 ± 0.03	12.27 ± 0.17	28.6 ± 10.9	1.47 ± 0.01
25	2.76 ± 0.02	12.83 ± 0.11	62.7 ± 6.4	1.46 ± 0.01
26	5.25 ± 0.02	12.54 ± 0.08	92.9 ± 5.9	1.42 ± 0.01
27	-1.94 ± 0.02	12.72 ± 0.09	64.8 ± 5.9	1.43 ± 0.01
28	6.70 ± 0.03	11.91 ± 0.09	204.9 ± 11.4	1.44 ± 0.01
29	5.85 ± 0.02	12.24 ± 0.09	163.1 ± 9.3	1.41 ± 0.01
30	1.52 ± 0.02	12.66 ± 0.08	88.2 ± 6.8	1.42 ± 0.01
32	0.35 ± 0.02	11.04 ± 0.10	28.4 ± 6.2	1.38 ± 0.01
33	4.28 ± 0.01	11.85 ± 0.07	85.8 ± 4.8	1.48 ± 0.01
34	0.77 ± 0.01	12.24 ± 0.07	17.8 ± 2.8	1.39 ± 0.01
35	-1.29 ± 0.01	11.26 ± 0.07	28.4 ± 3.9	1.46 ± 0.01
36	0.28 ± 0.03	12.74 ± 0.18	7.1 ± 8.4	1.43 ± 0.01
37	4.78 ± 0.02	12.85 ± 0.09	100.7 ± 6.3	1.38 ± 0.01
38	2.43 ± 0.01	13.01 ± 0.09	18.9 ± 4.5	1.41 ± 0.01
39	1.49 ± 0.01	12.71 ± 0.10	30.1 ± 4.5	1.43 ± 0.01
40	-0.97 ± 0.02	14.08 ± 0.09	14.6 ± 4.7	1.42 ± 0.01
41	6.15 ± 0.02	13.95 ± 0.09	103.4 ± 6.5	1.36 ± 0.01
42	6.23 ± 0.03	12.69 ± 0.11	165.0 ± 11.1	1.41 ± 0.01
43	12.46 ± 0.03	12.71 ± 0.12	217.1 ± 14.6	1.41 ± 0.01
44	3.44 ± 0.04	13.57 ± 0.12	197.0 ± 13.6	1.38 ± 0.01
45	3.80 ± 0.02	13.16 ± 0.07	161.9 ± 7.4	1.36 ± 0.01
46	0.42 ± 0.02	10.92 ± 0.07	28.2 ± 4.7	1.17 ± 0.01
48	2.27 ± 0.02	12.06 ± 0.10	29.0 ± 4.2	1.46 ± 0.01
50	8.20 ± 0.01	12.18 ± 0.08	70.1 ± 4.7	1.44 ± 0.01
51	1.53 ± 0.02	13.06 ± 0.09	59.5 ± 6.4	1.48 ± 0.01
52	8.42 ± 0.02	14.19 ± 0.21	21.9 ± 8.6	1.41 ± 0.01
53	-2.80 ± 0.01	10.83 ± 0.07	47.6 ± 4.4	1.33 ± 0.01
54	3.25 ± 0.01	10.51 ± 0.06	69.5 ± 3.8	1.32 ± 0.01

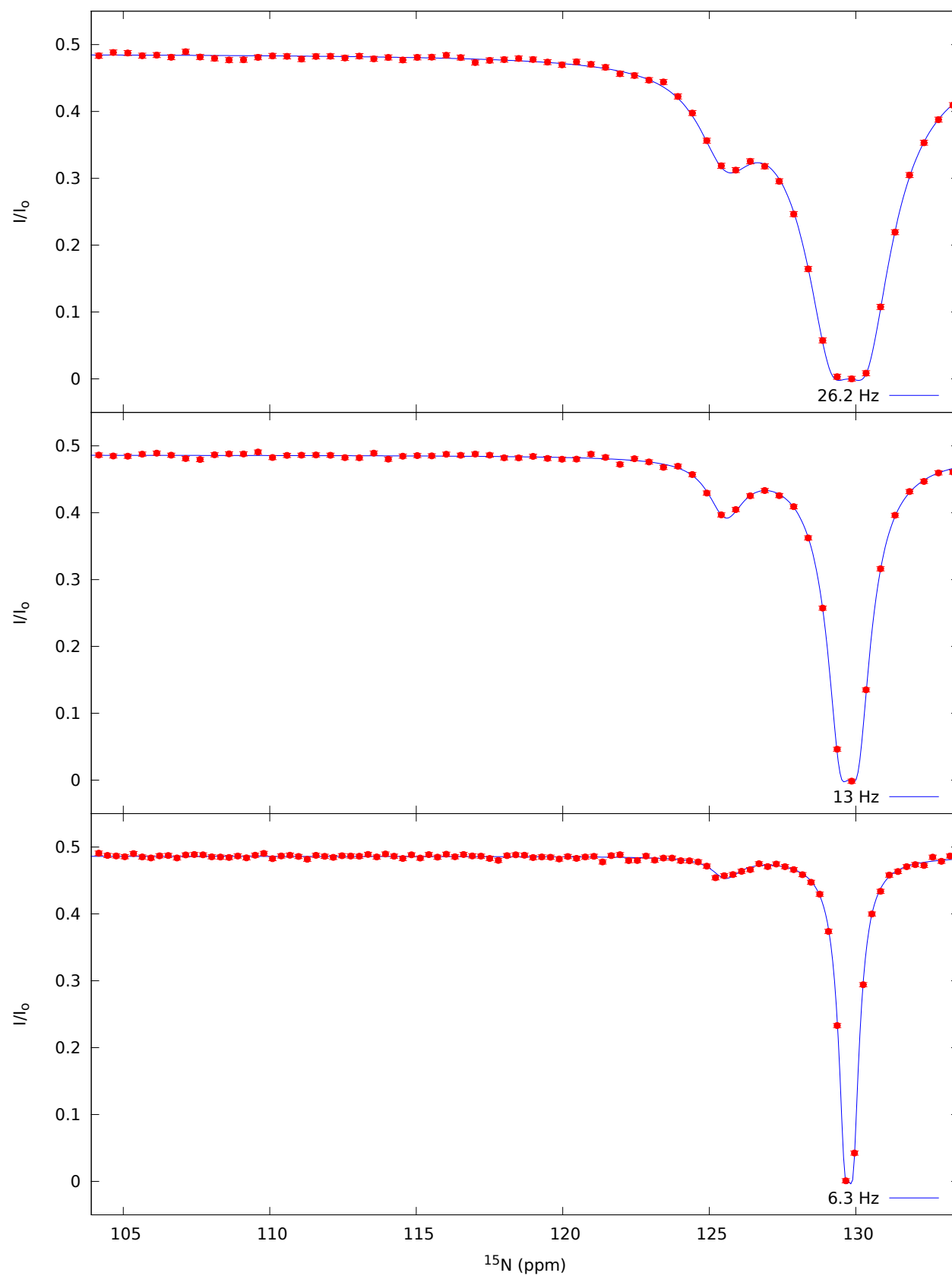
55	-6.38 ± 0.02	11.84 ± 0.10	35.0 ± 4.4	1.45 ± 0.01
56	3.64 ± 0.02	12.13 ± 0.09	88.1 ± 6.3	1.41 ± 0.01
57	0.56 ± 0.02	12.68 ± 0.11	38.2 ± 7.2	1.39 ± 0.01
58	0.87 ± 0.03	12.30 ± 0.14	40.3 ± 8.8	1.47 ± 0.01
59	6.30 ± 0.01	12.77 ± 0.12	38.5 ± 5.9	1.43 ± 0.01
60	2.40 ± 0.01	12.64 ± 0.06	33.9 ± 3.2	1.38 ± 0.01
61	2.22 ± 0.01	12.91 ± 0.08	18.8 ± 3.4	1.44 ± 0.01
62	-1.02 ± 0.01	12.39 ± 0.11	13.7 ± 4.5	1.47 ± 0.01
63	2.93 ± 0.01	12.33 ± 0.07	39.9 ± 4.0	1.43 ± 0.01
64	2.08 ± 0.01	12.49 ± 0.08	20.7 ± 3.4	1.41 ± 0.01
65	-1.64 ± 0.01	11.88 ± 0.09	21.7 ± 4.3	1.43 ± 0.01
66	4.18 ± 0.01	12.24 ± 0.09	49.5 ± 5.2	1.45 ± 0.01
67	8.04 ± 0.01	12.16 ± 0.07	62.0 ± 3.8	1.39 ± 0.01
68	4.38 ± 0.01	10.53 ± 0.05	44.0 ± 2.9	1.46 ± 0.01
69	1.85 ± 0.01	7.70 ± 0.04	12.5 ± 2.0	1.56 ± 0.01
70	0.57 ± 0.01	6.73 ± 0.03	3.1 ± 1.6	1.54 ± 0.01
71	-0.18 ± 0.01	4.34 ± 0.09	1.0 ± 5.0	1.50 ± 0.01

Supporting Figure 2. Intensity profiles (red) and best fits (blue) from the global fit of 37 residues of the A39G FF domain, 1°C, 11.7T, showing clear evidence of exchange.

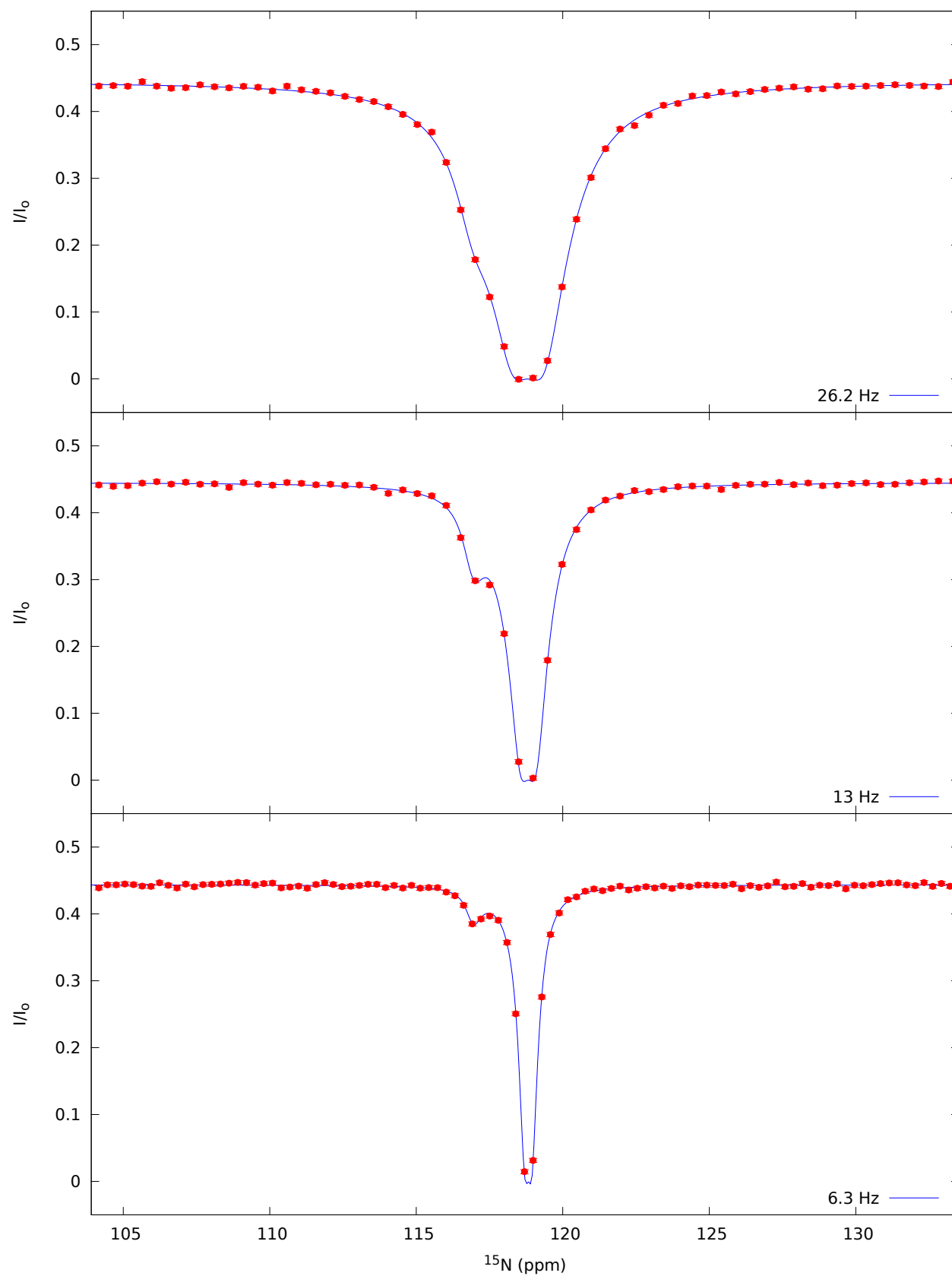
Residue 10



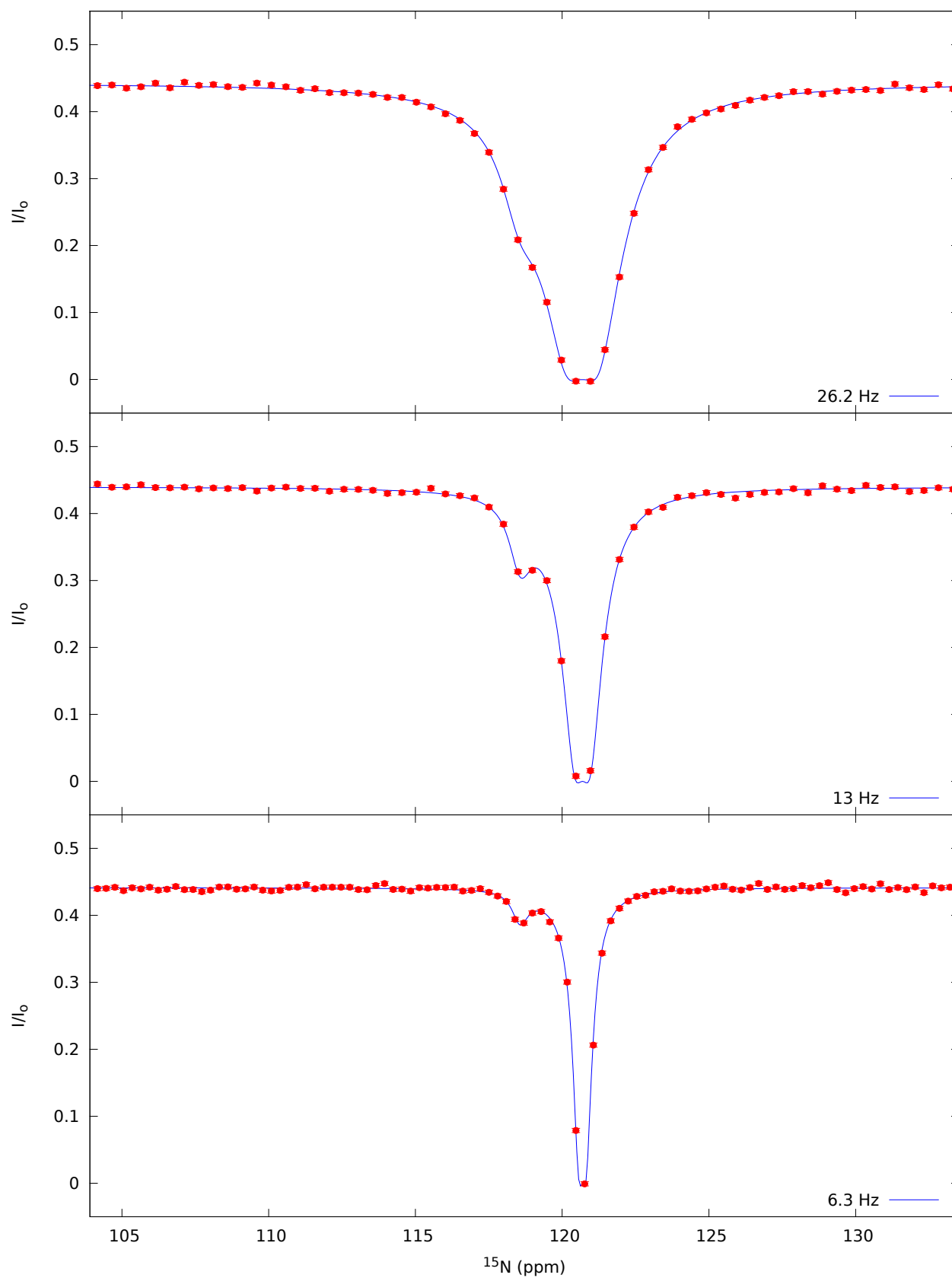
Residue 13



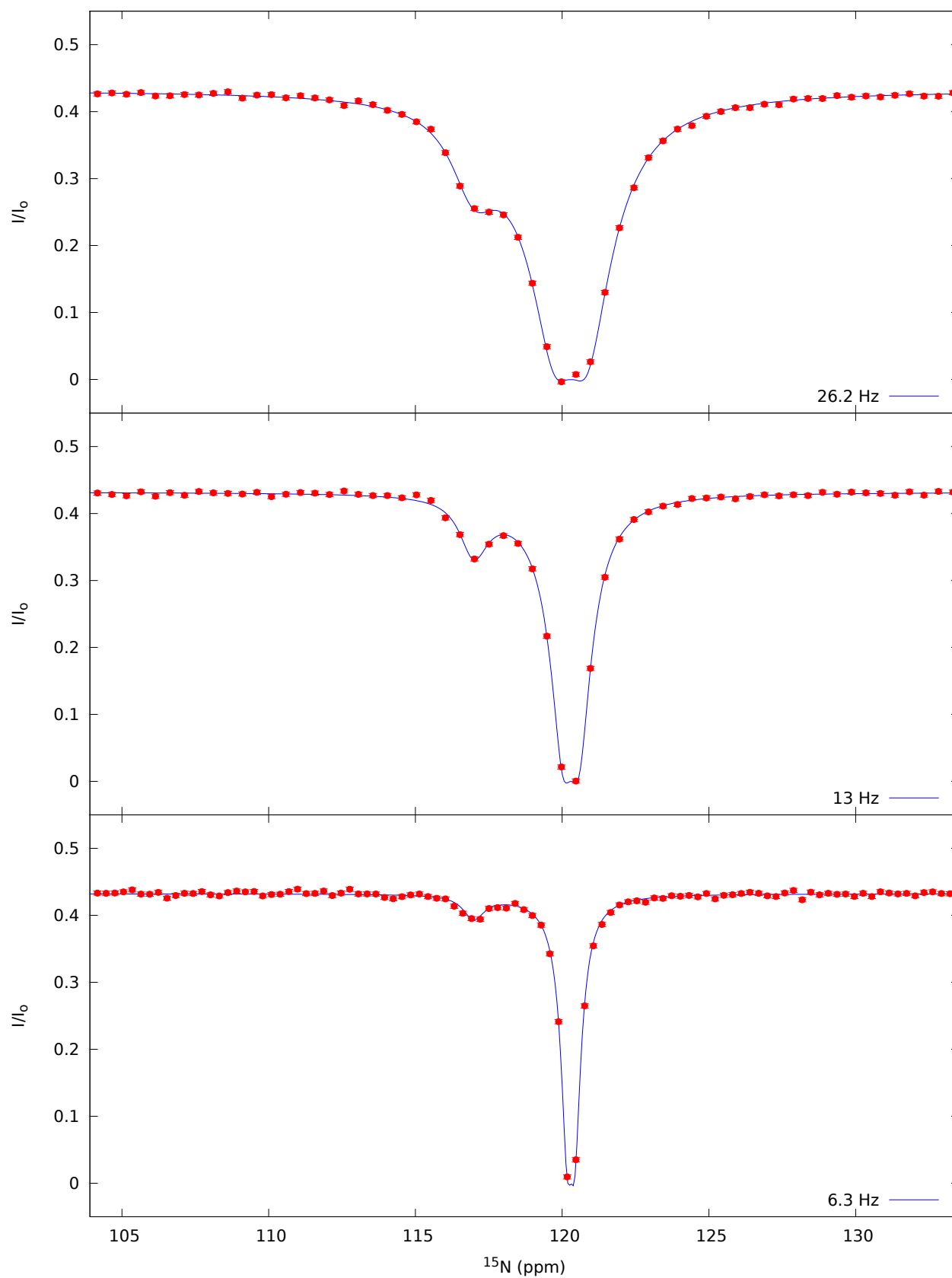
Residue 15



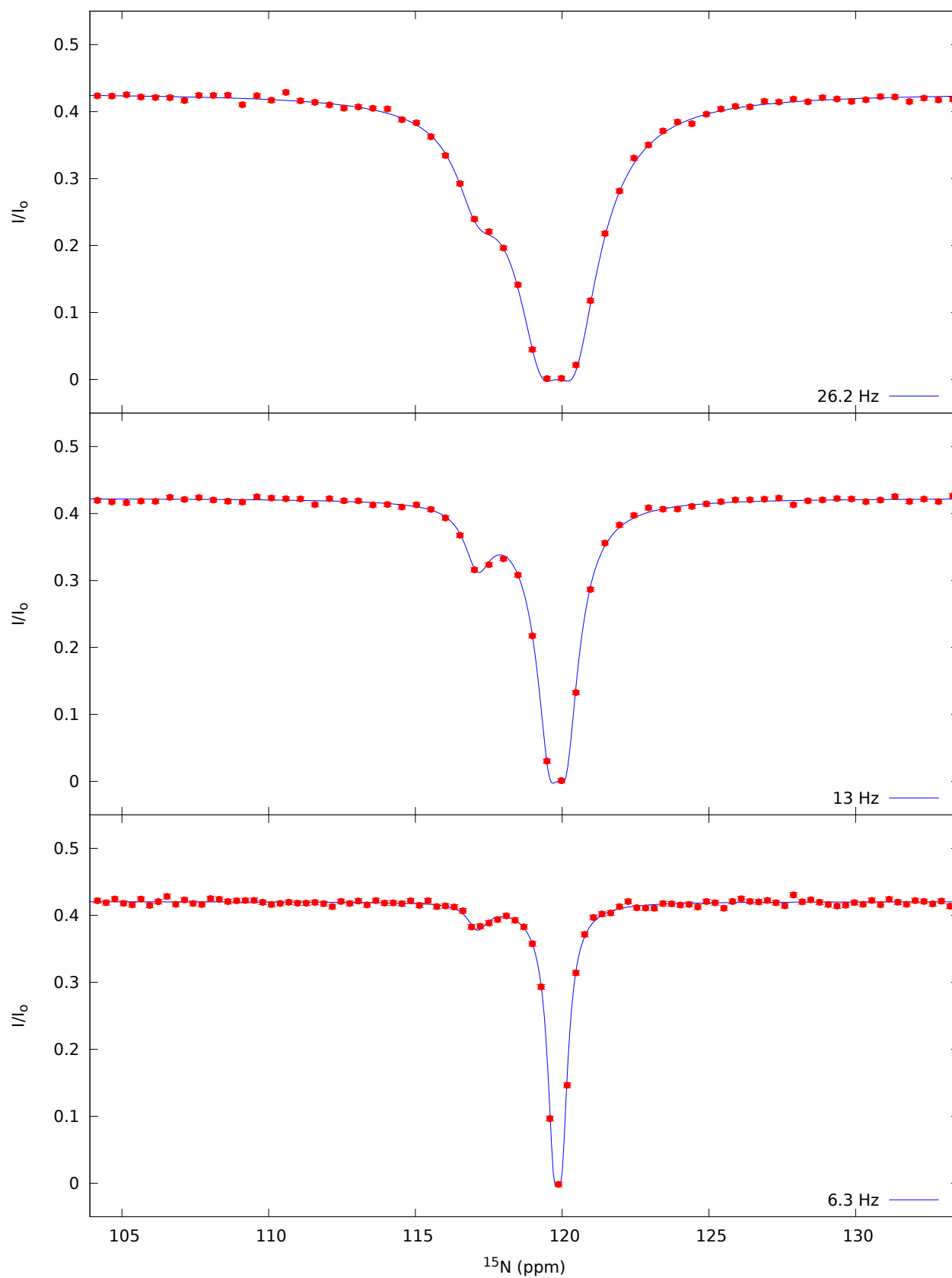
Residue 18



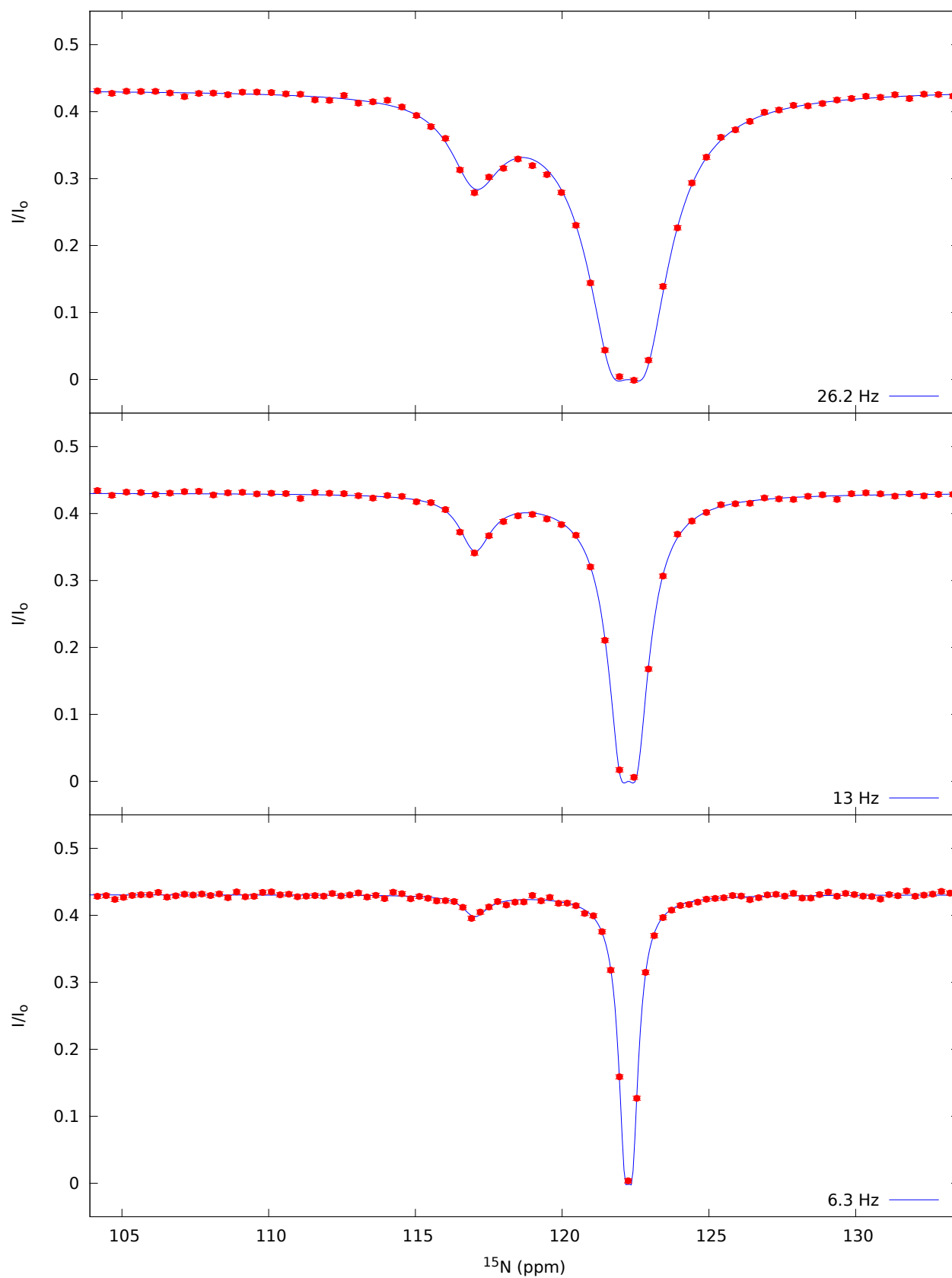
Residue 22



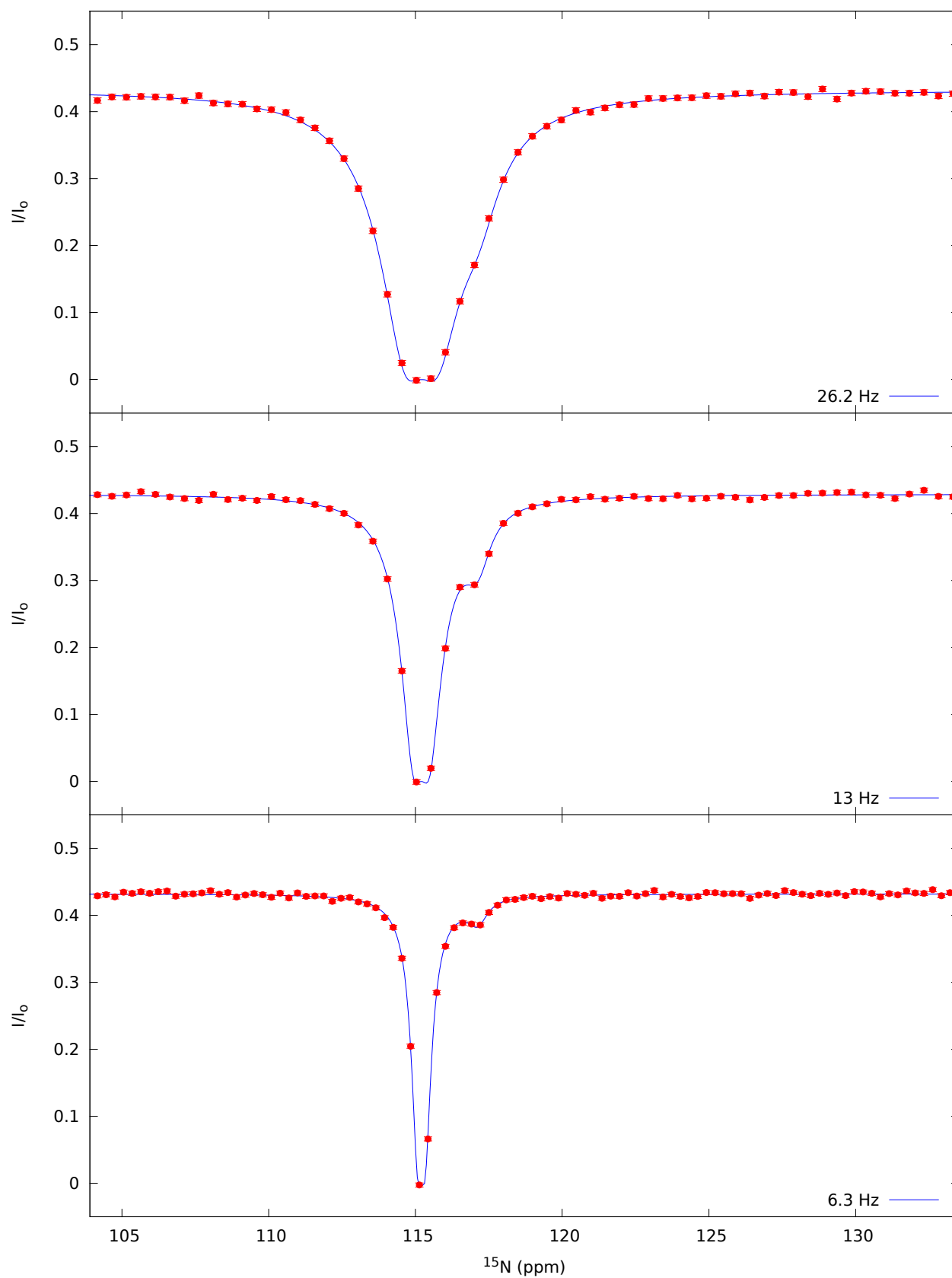
Residue 25



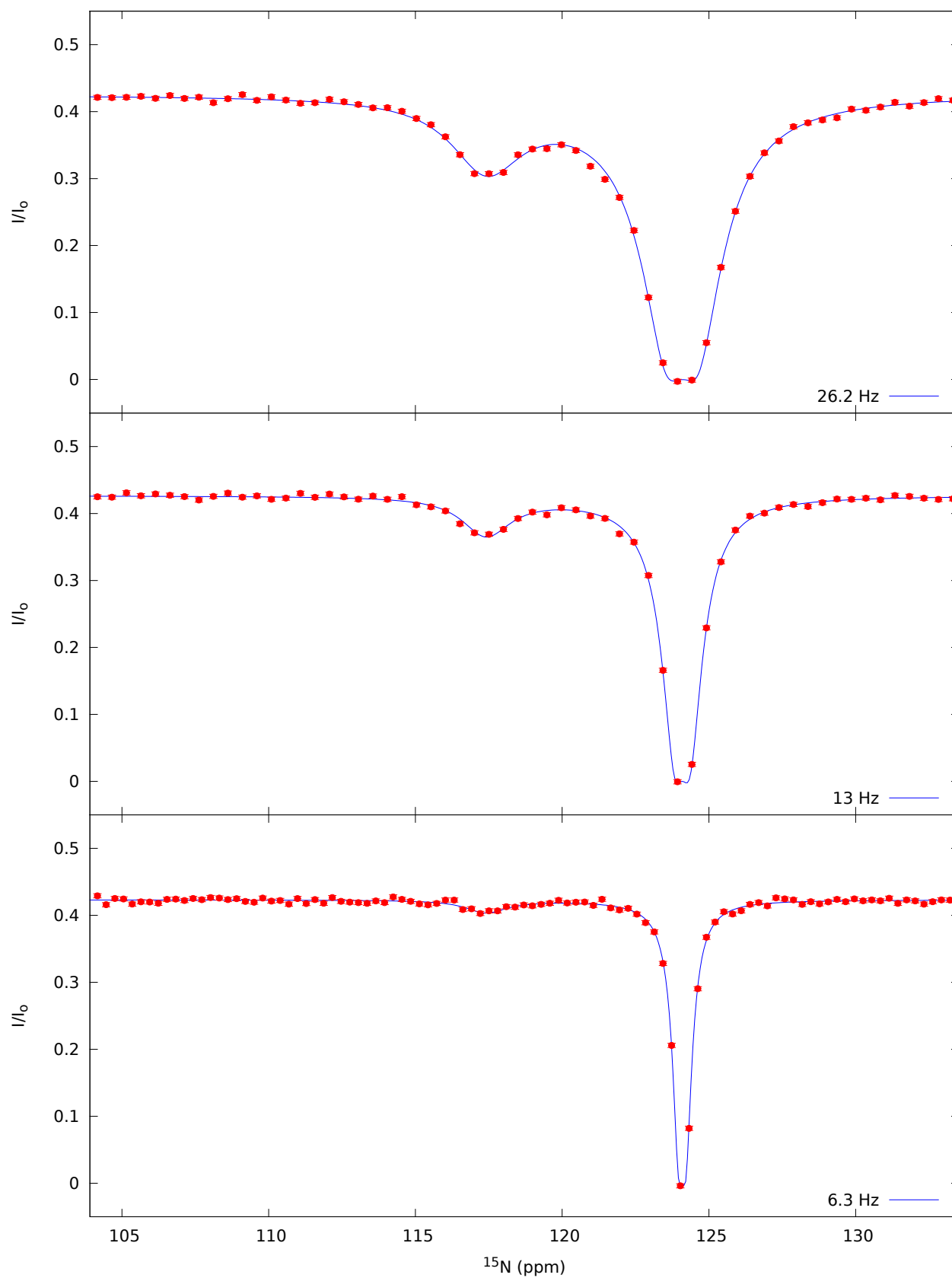
Residue 26



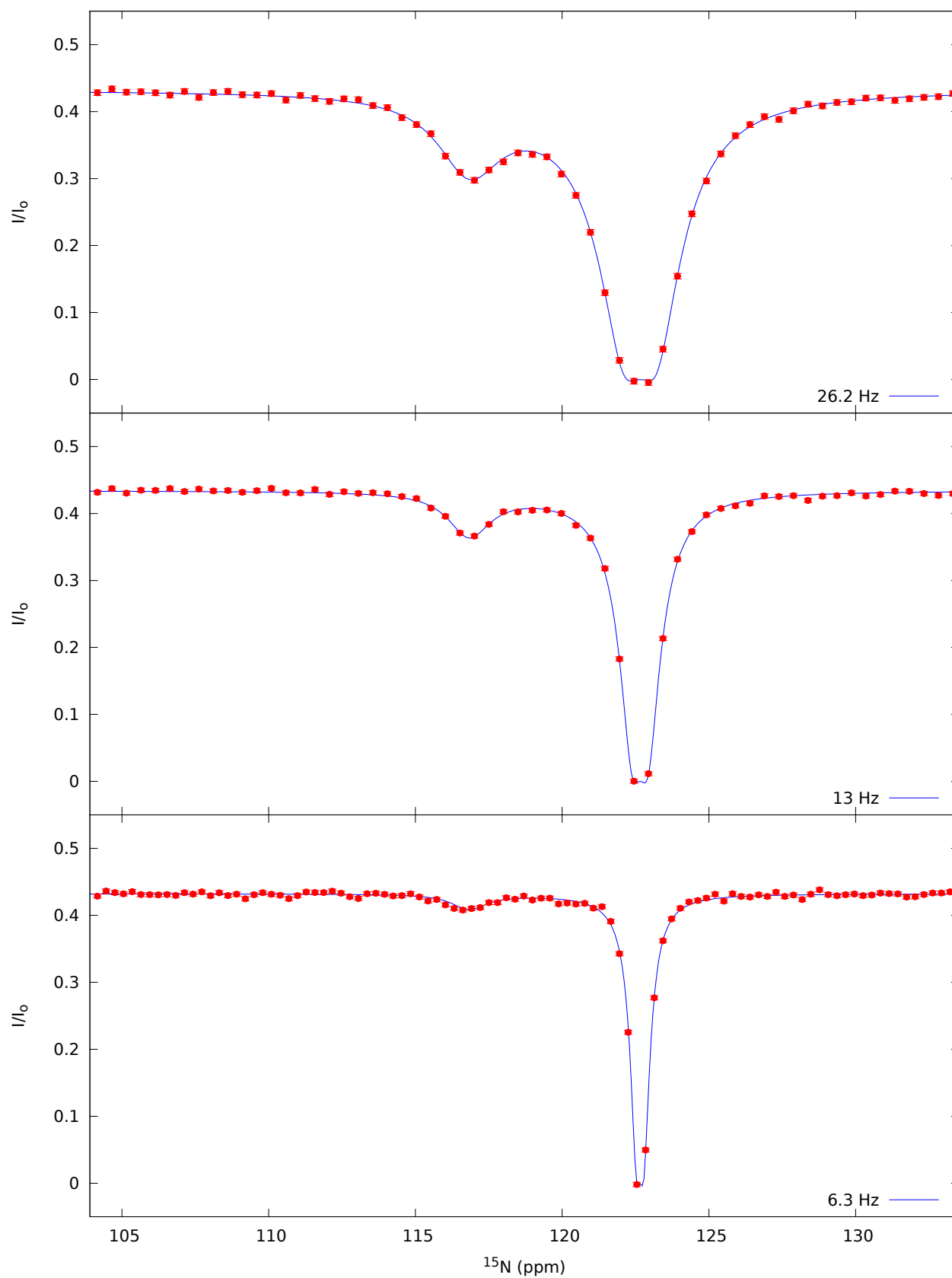
Residue 27



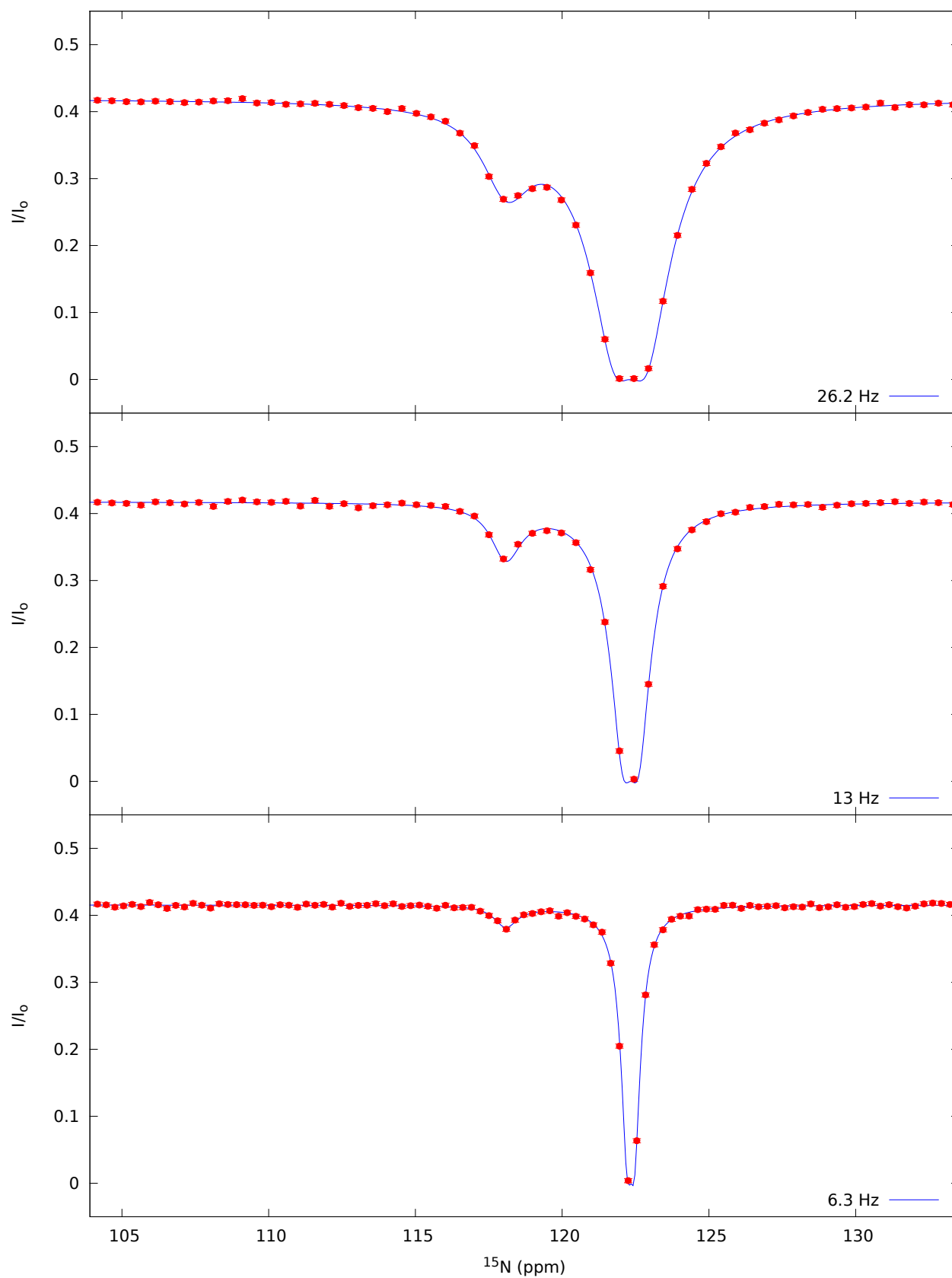
Residue 28



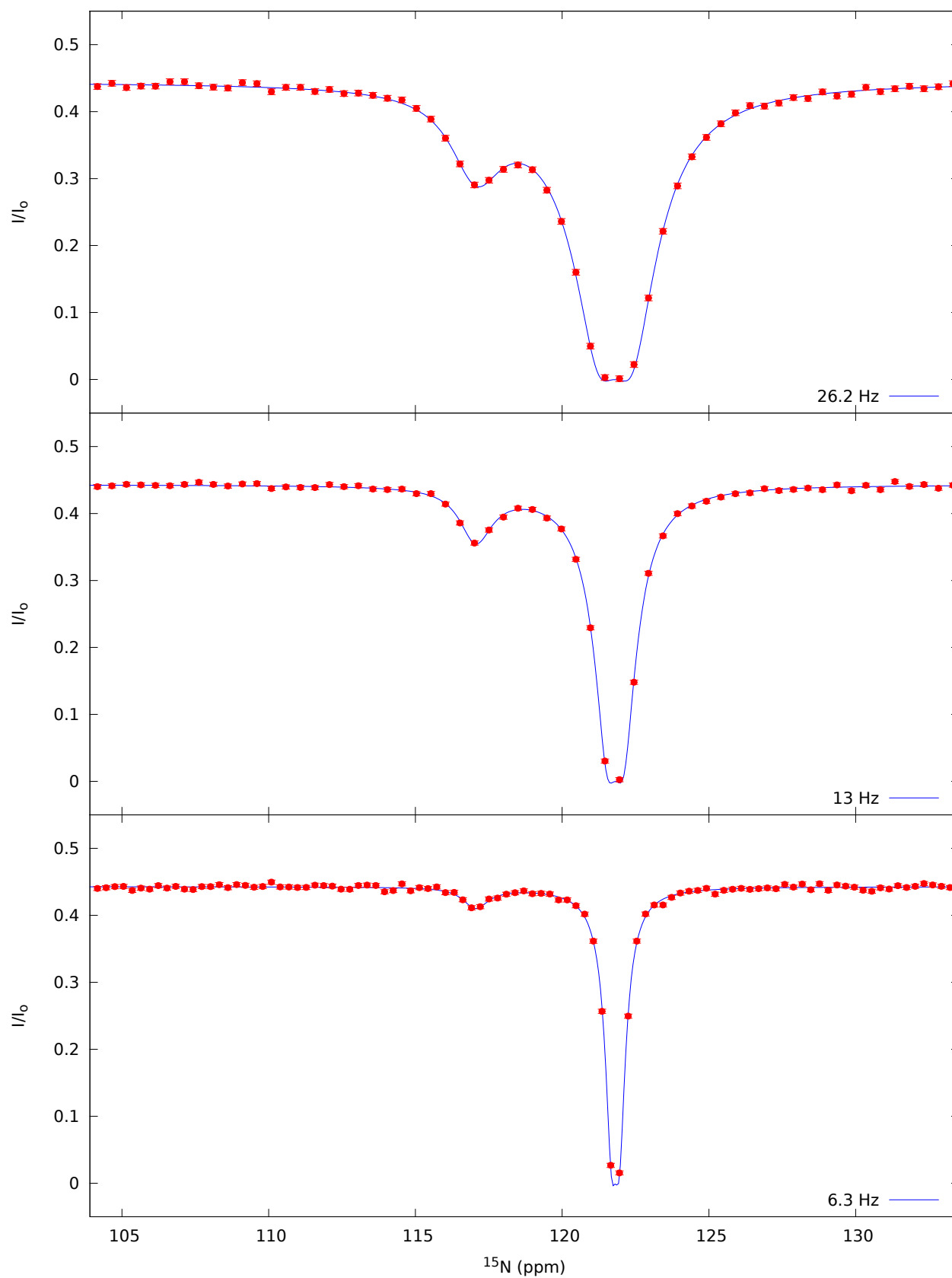
Residue 29



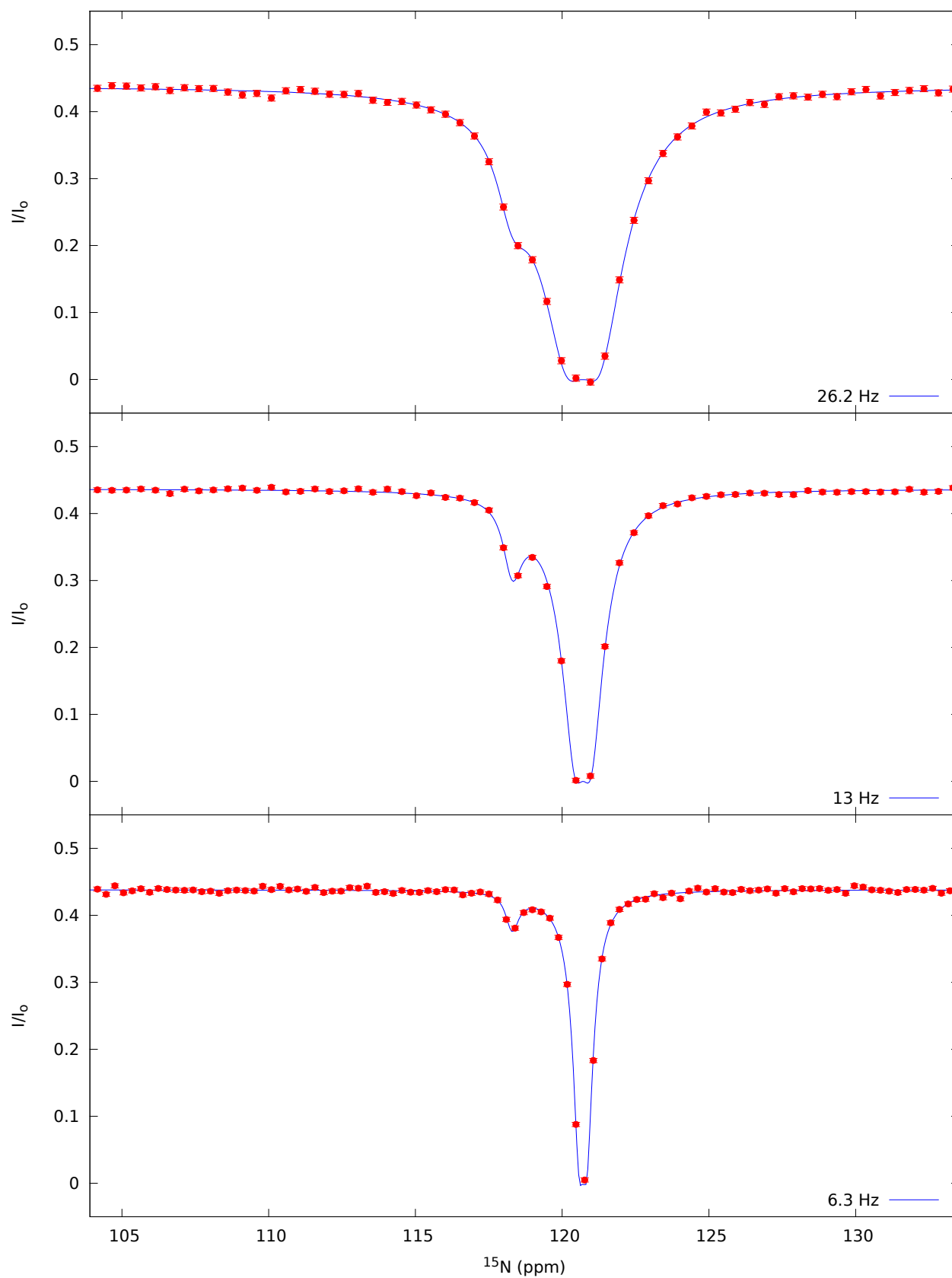
Residue 33



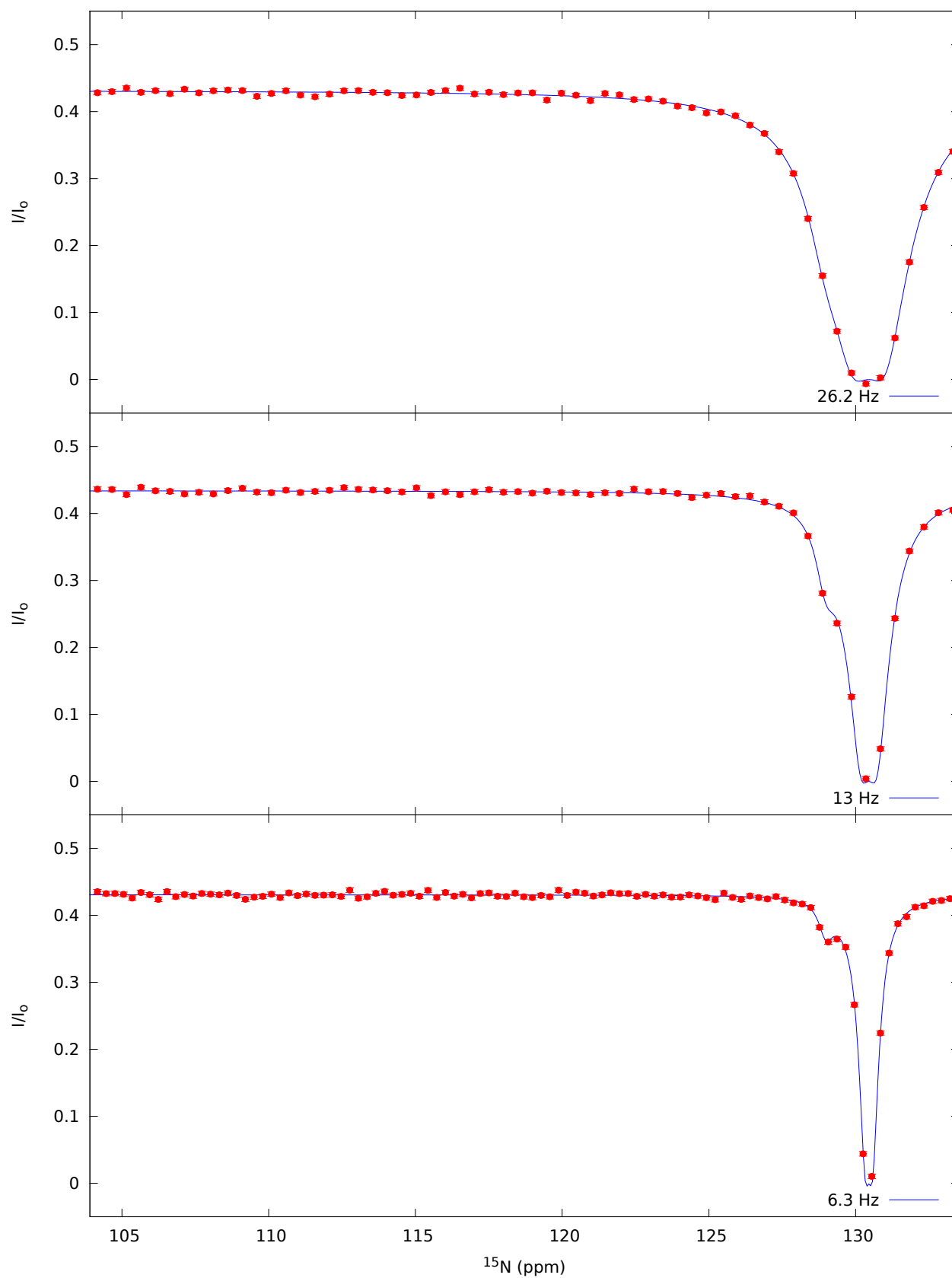
Residue 37



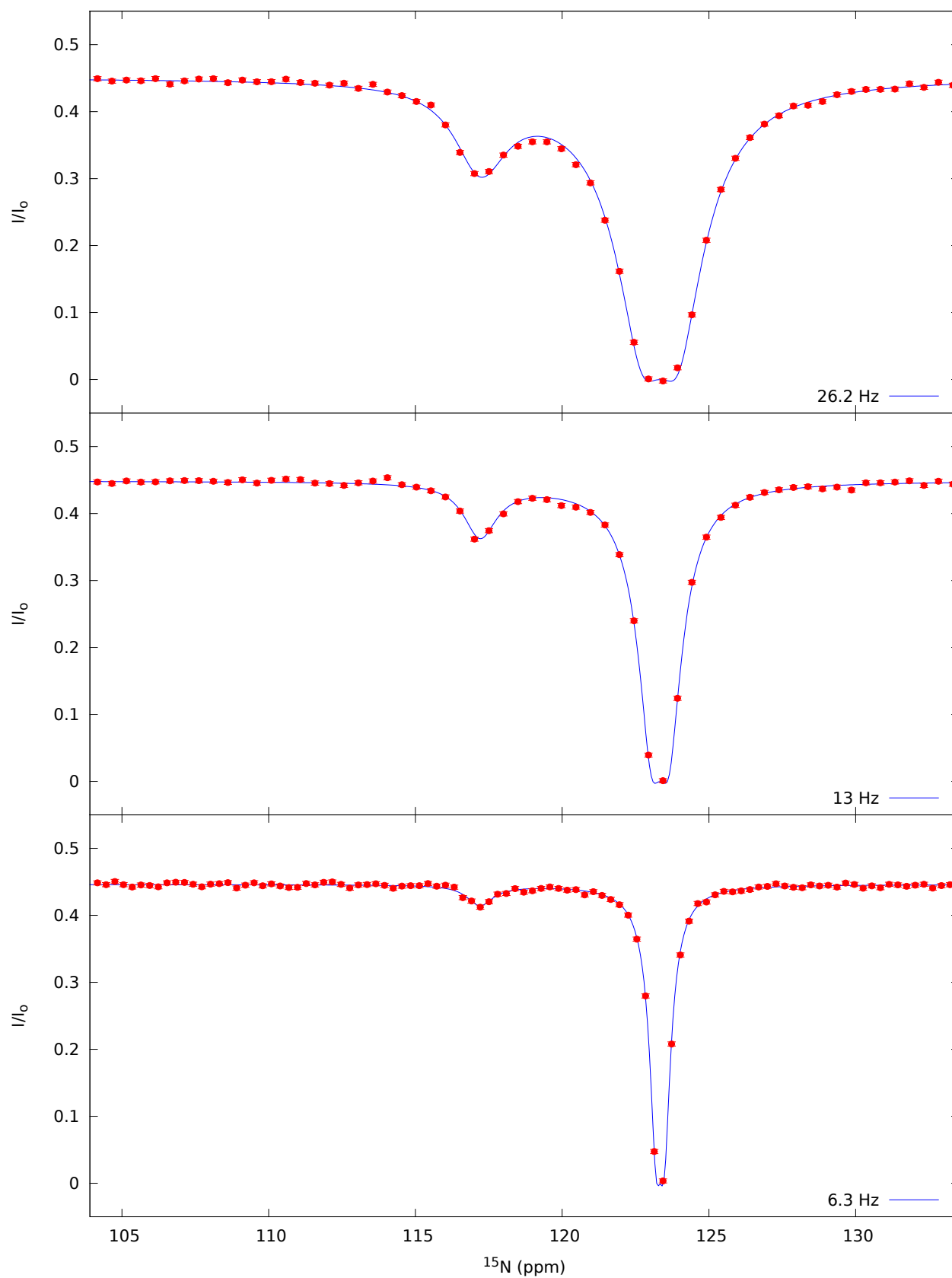
Residue 38



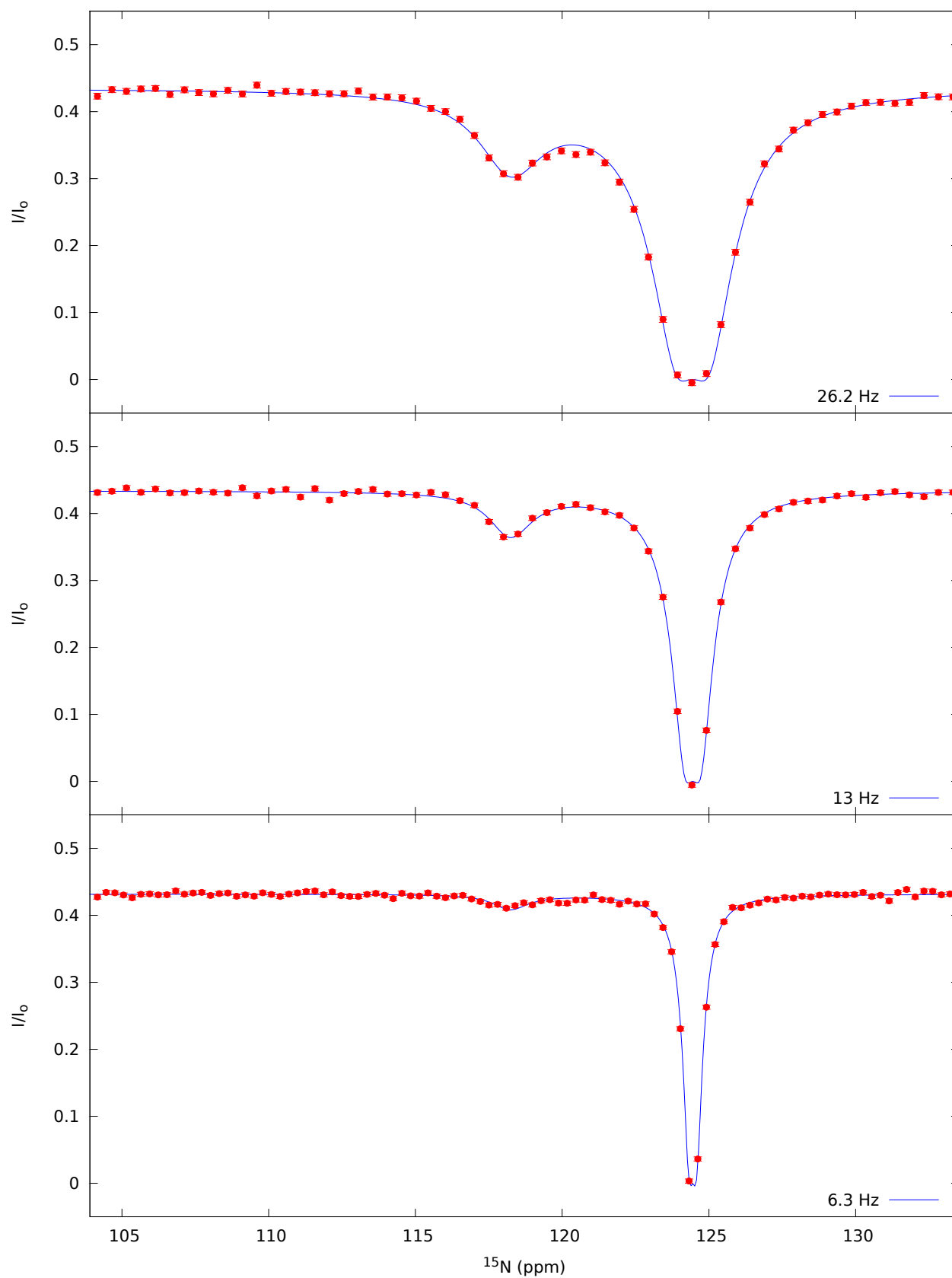
Residue 39



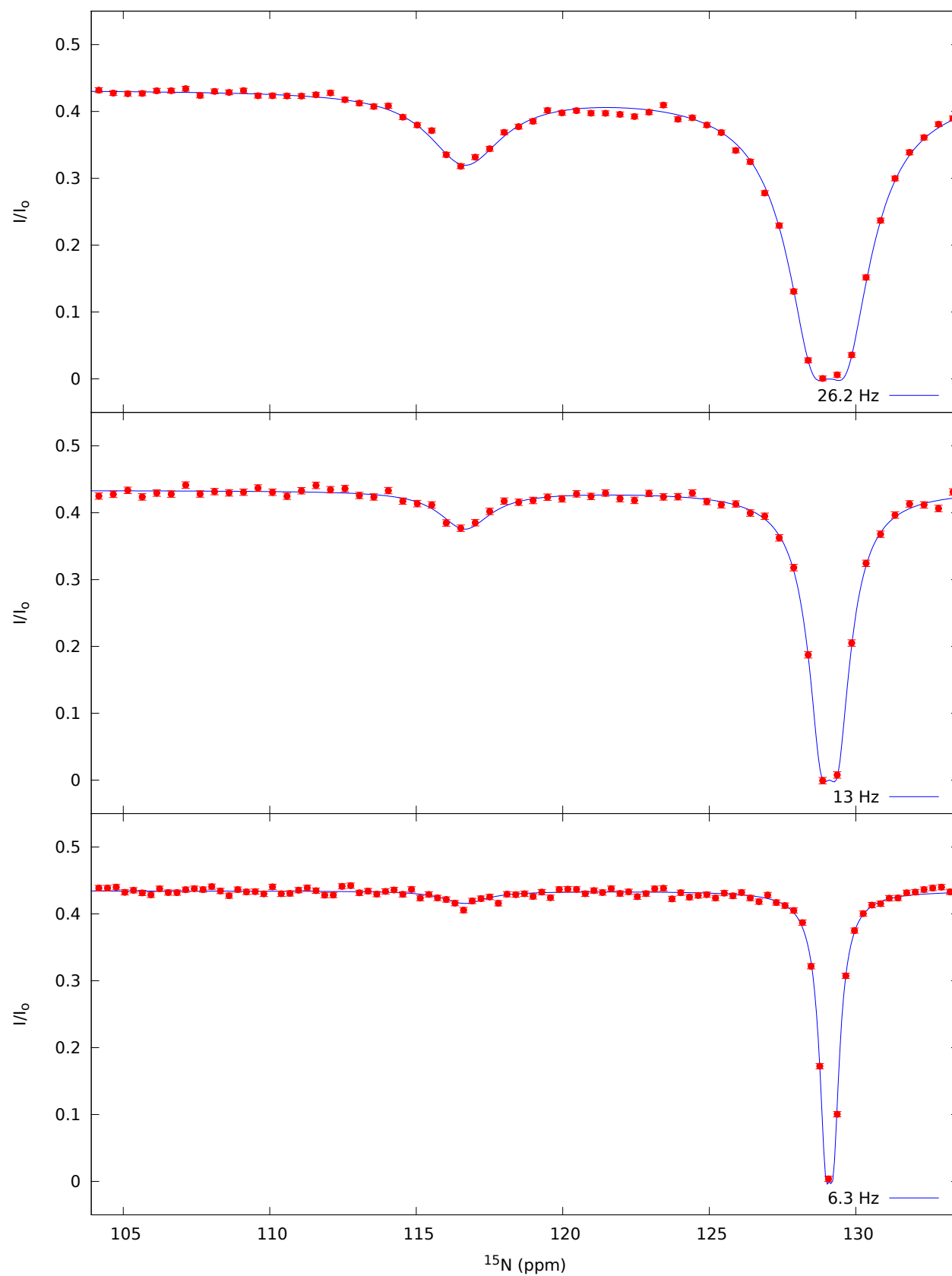
Residue 41



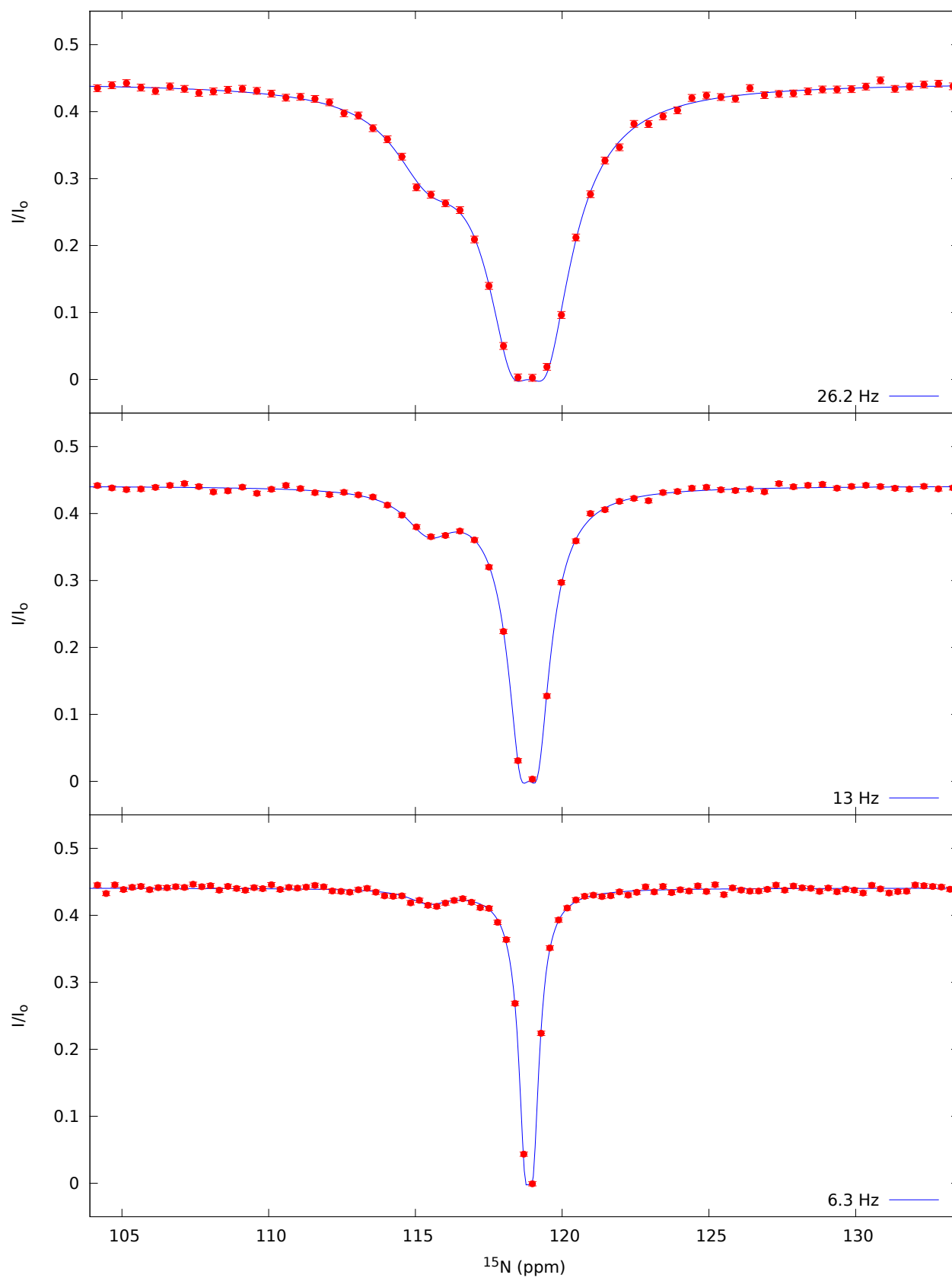
Residue 42



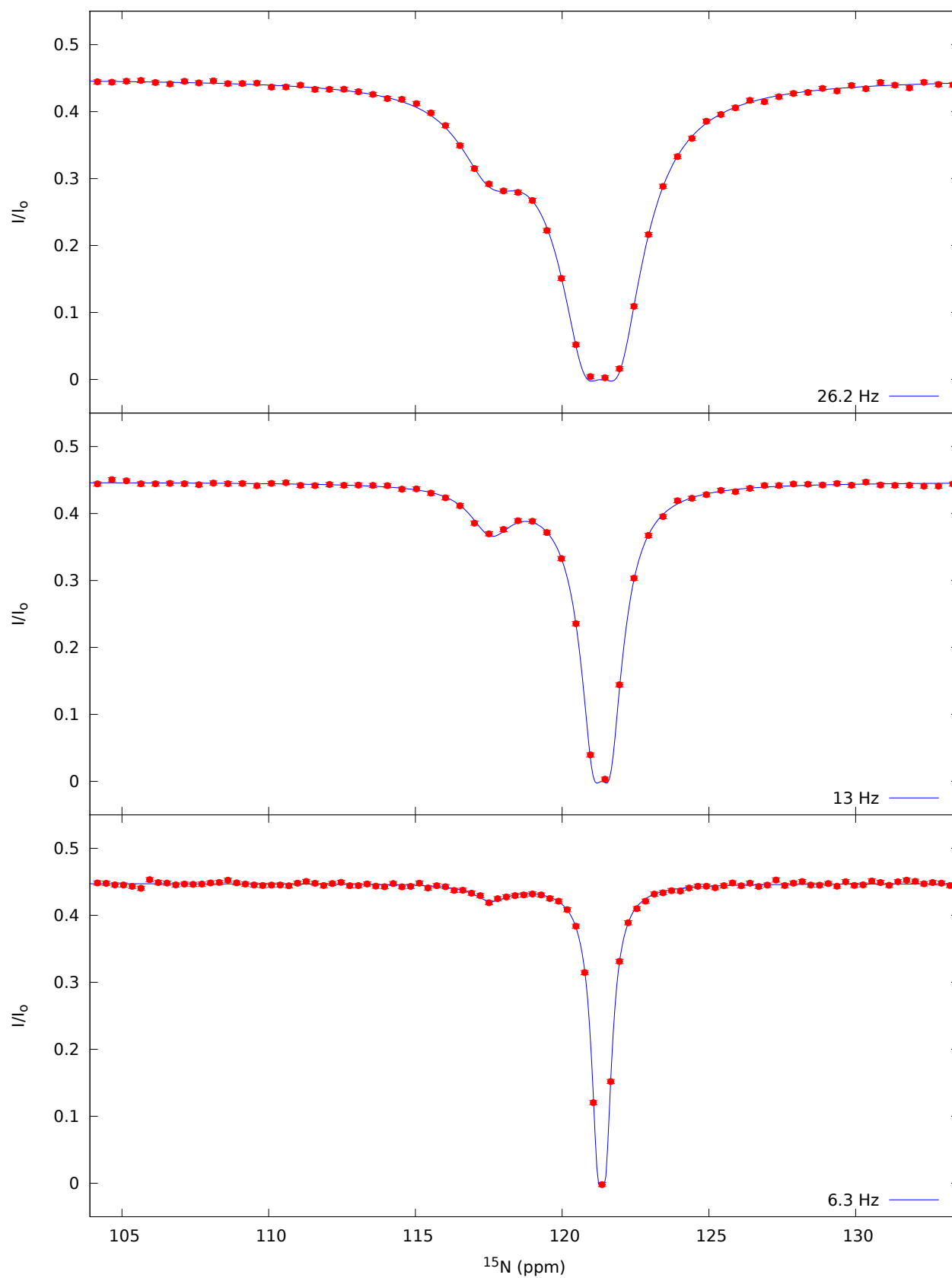
Residue 43



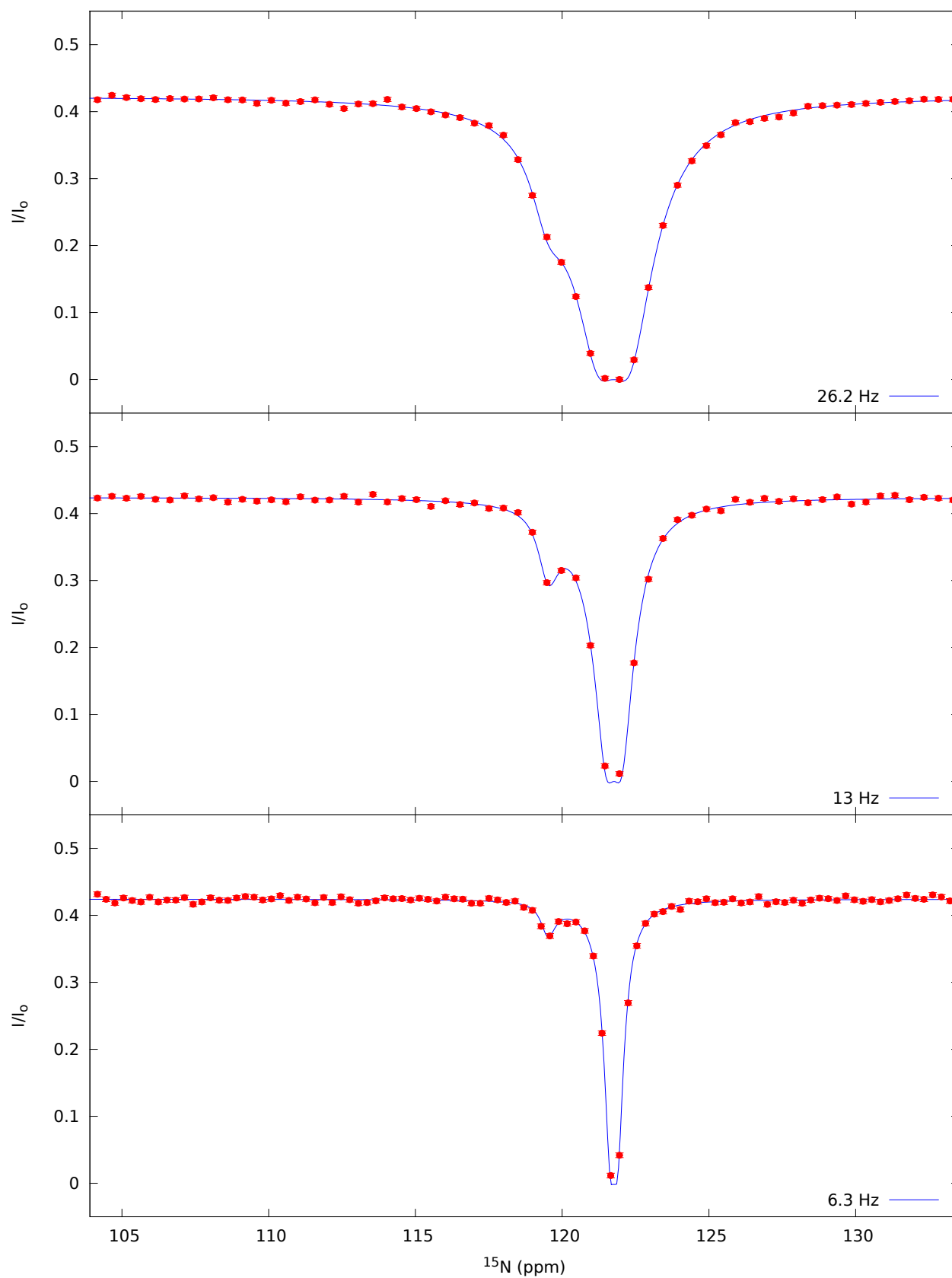
Residue 44



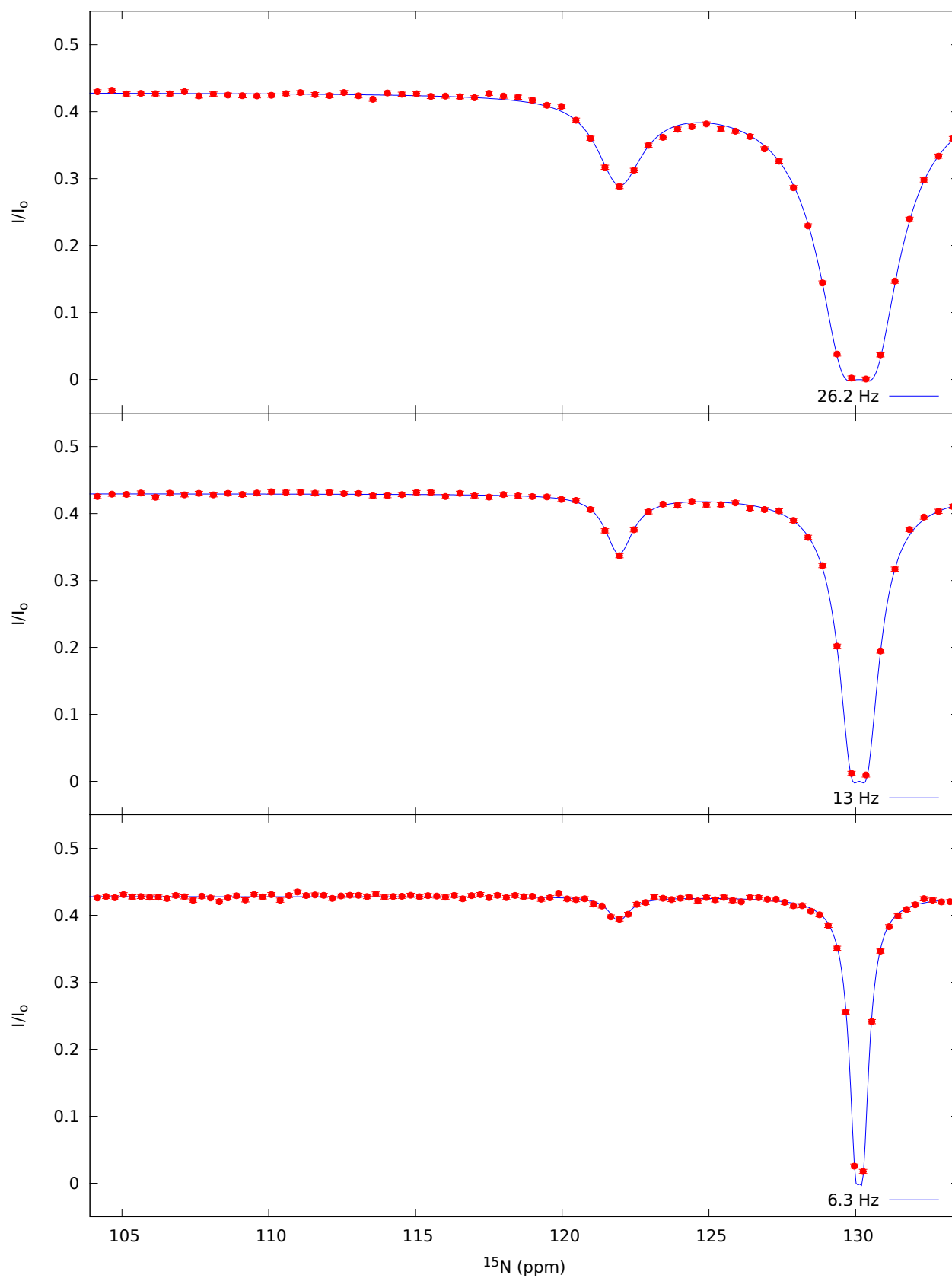
Residue 45



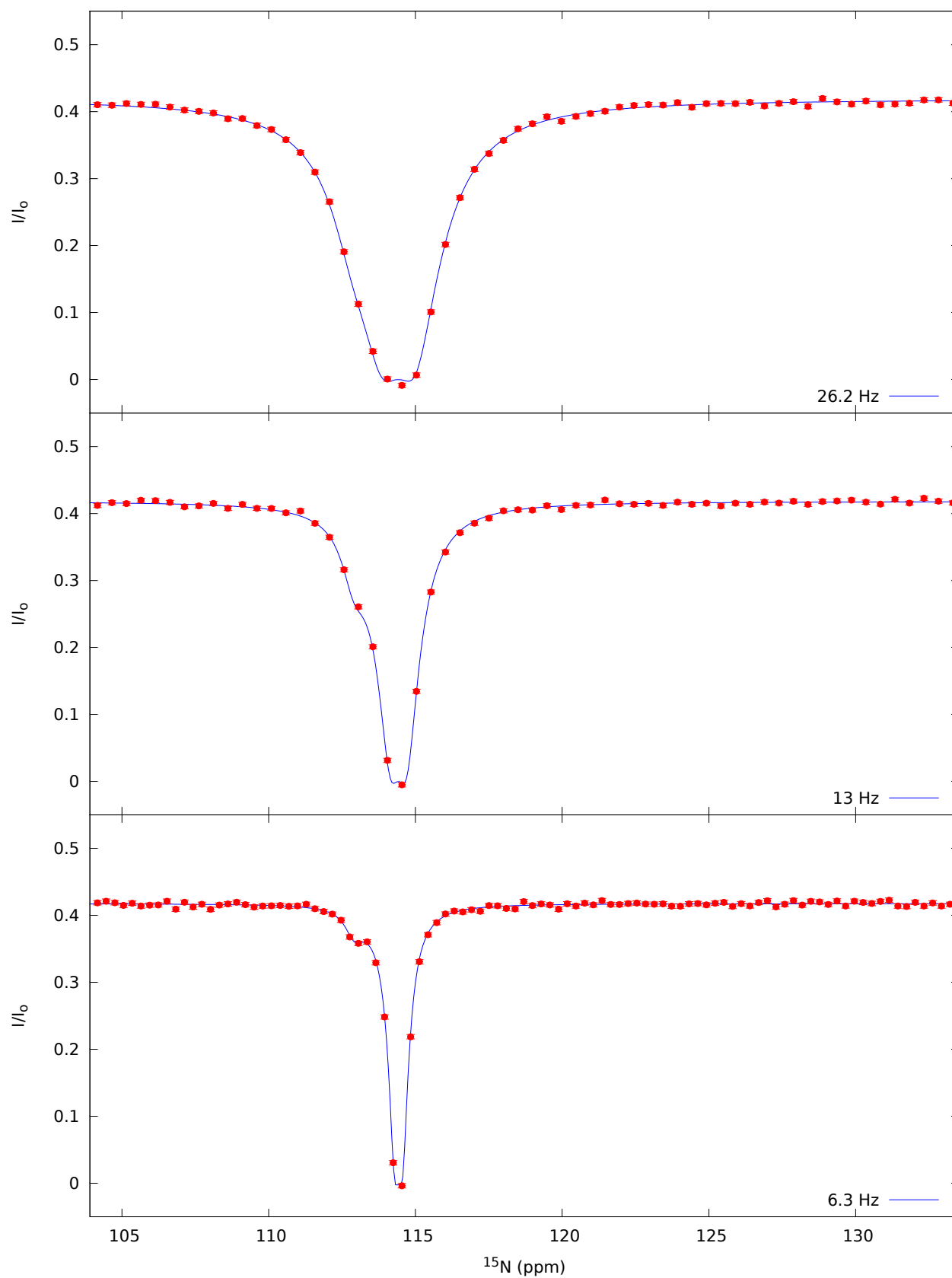
Residue 48



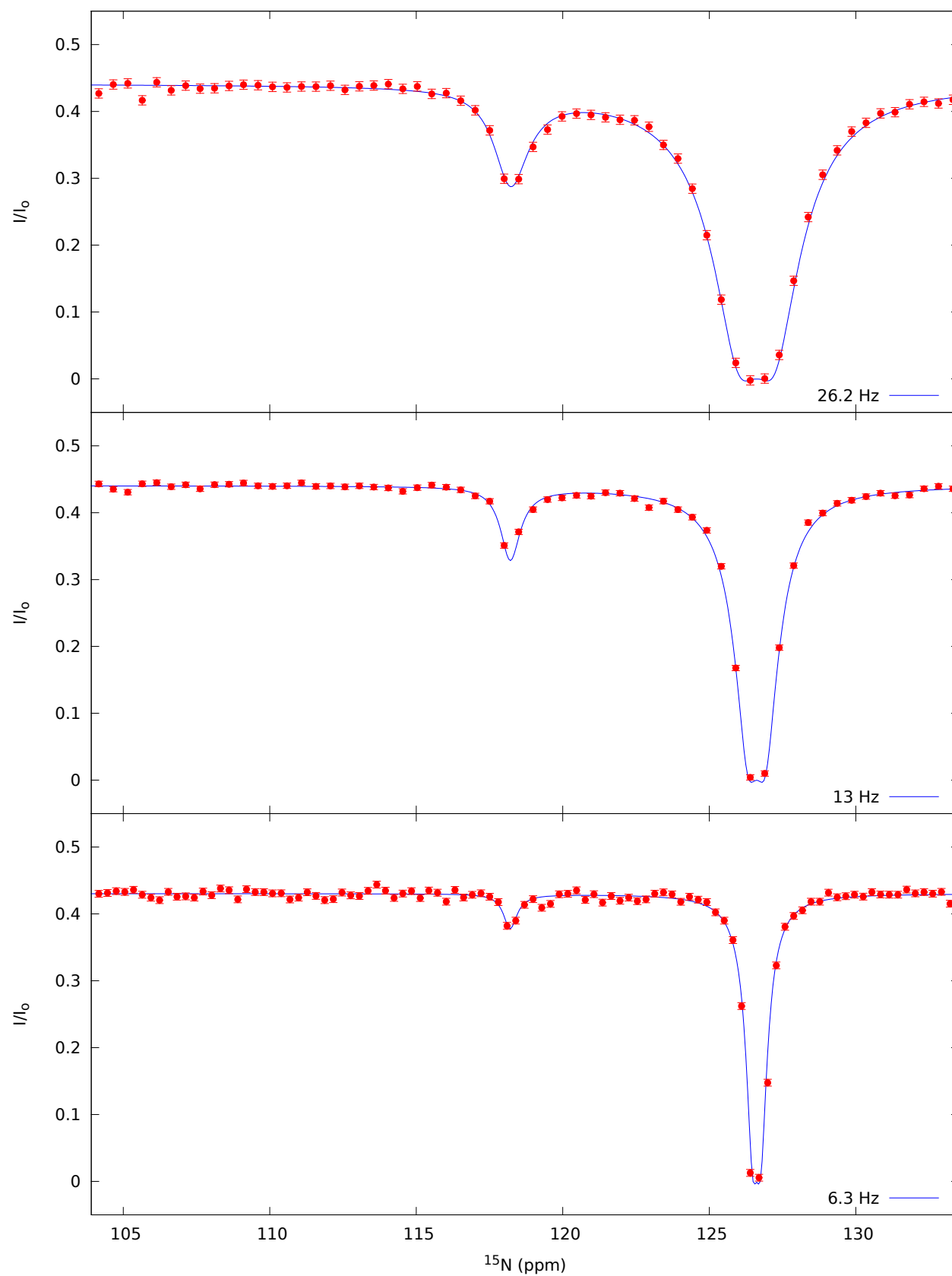
Residue 50



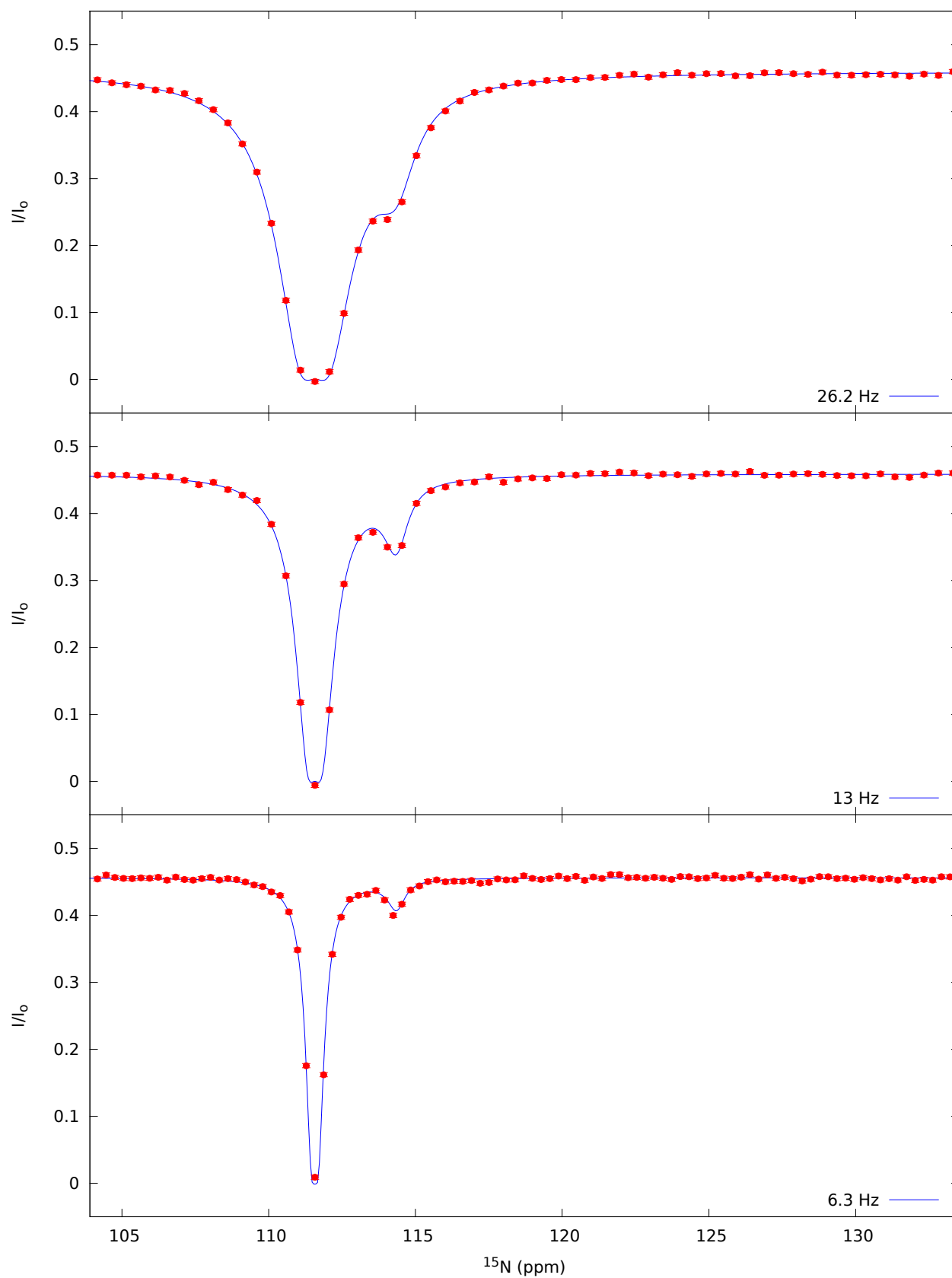
Residue 51



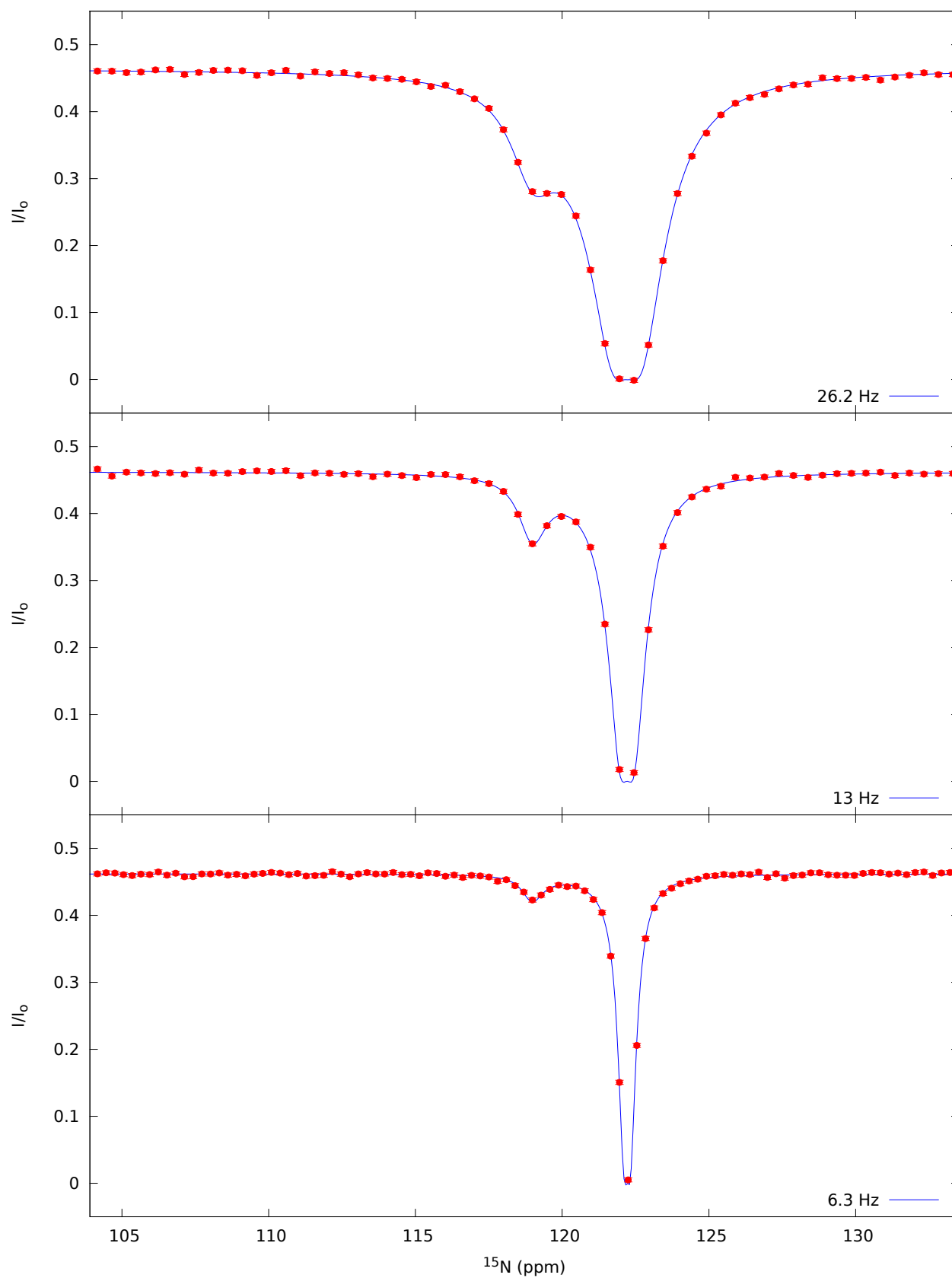
Residue 52



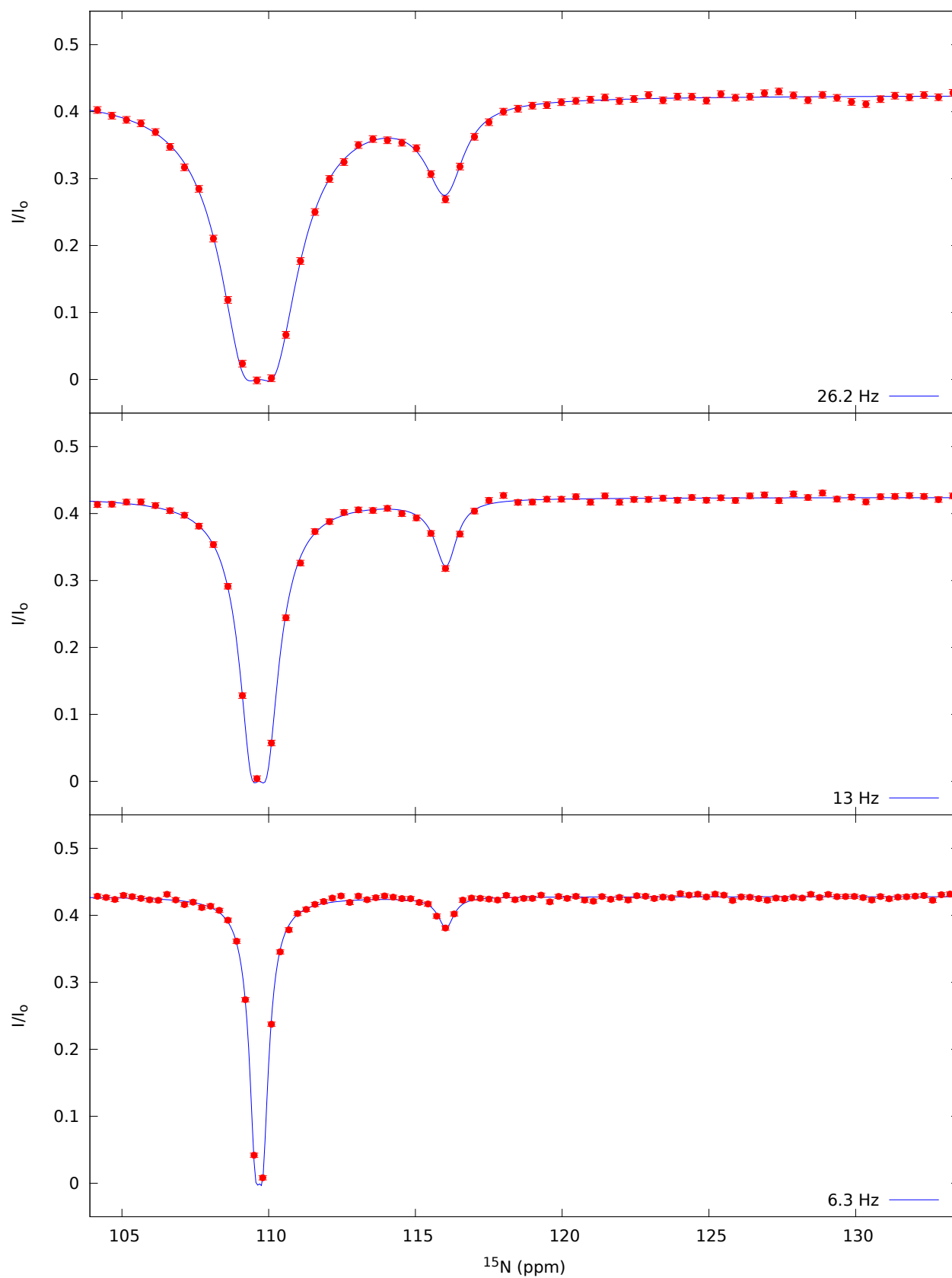
Residue 53



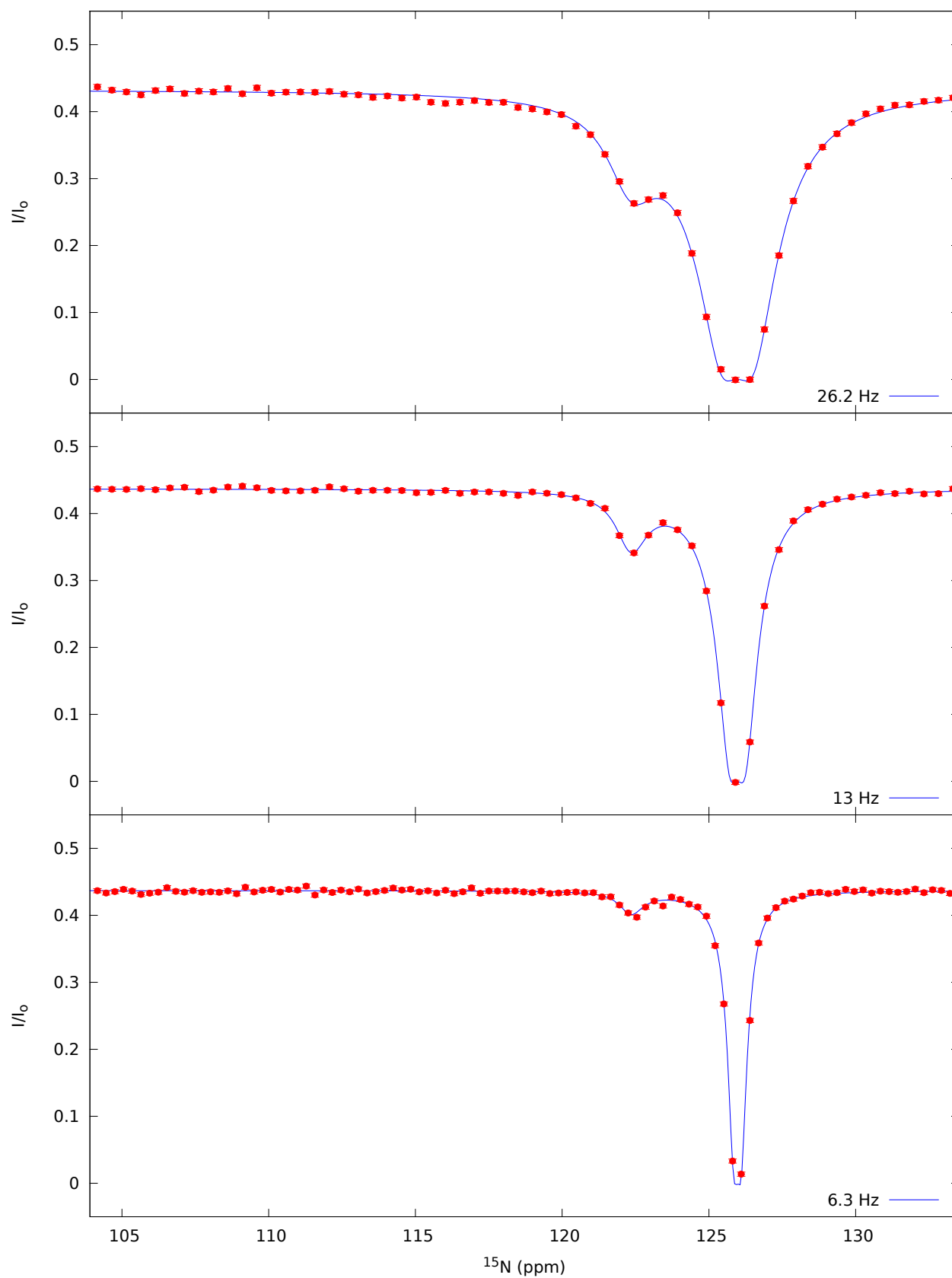
Residue 54



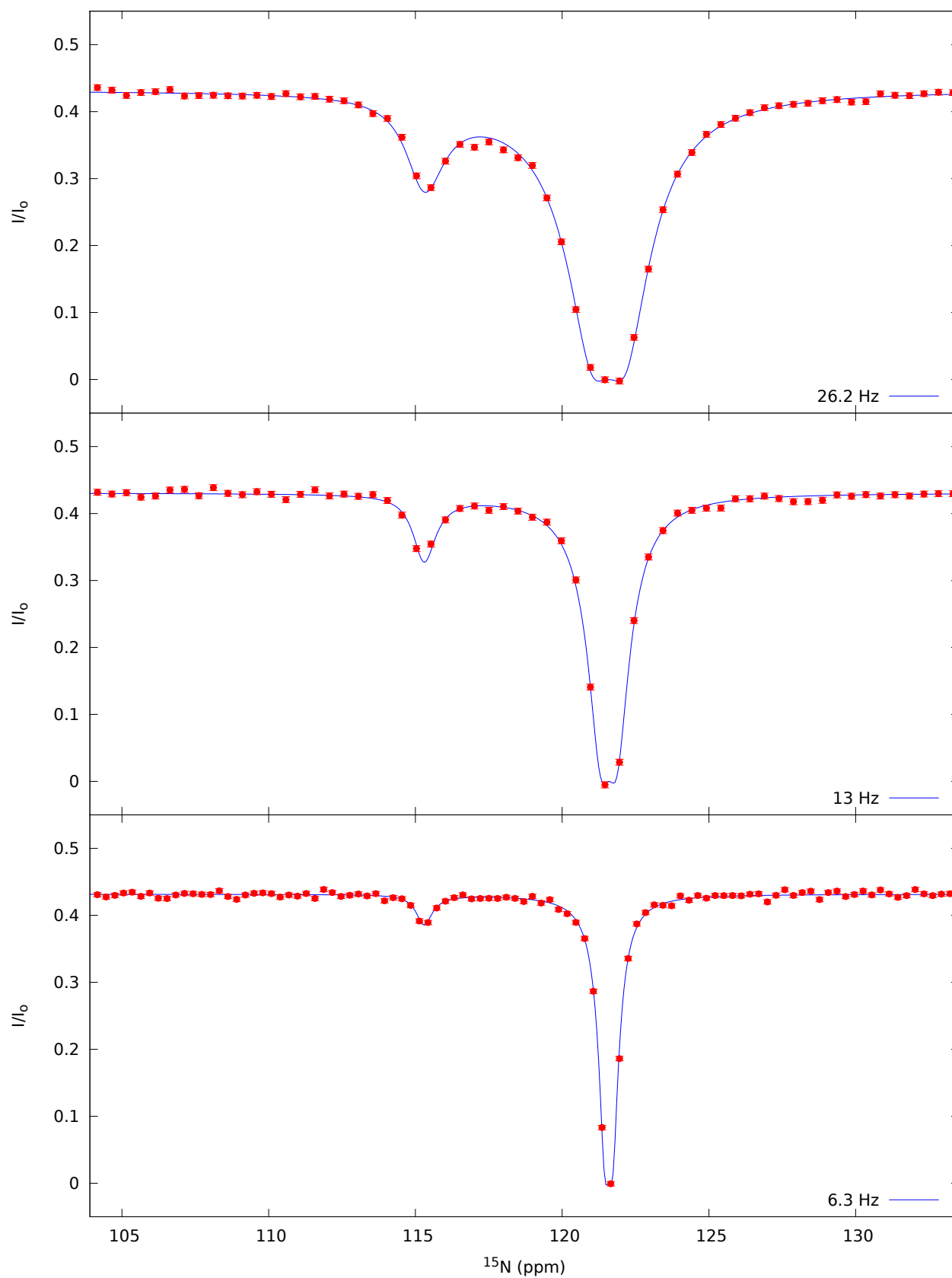
Residue 55



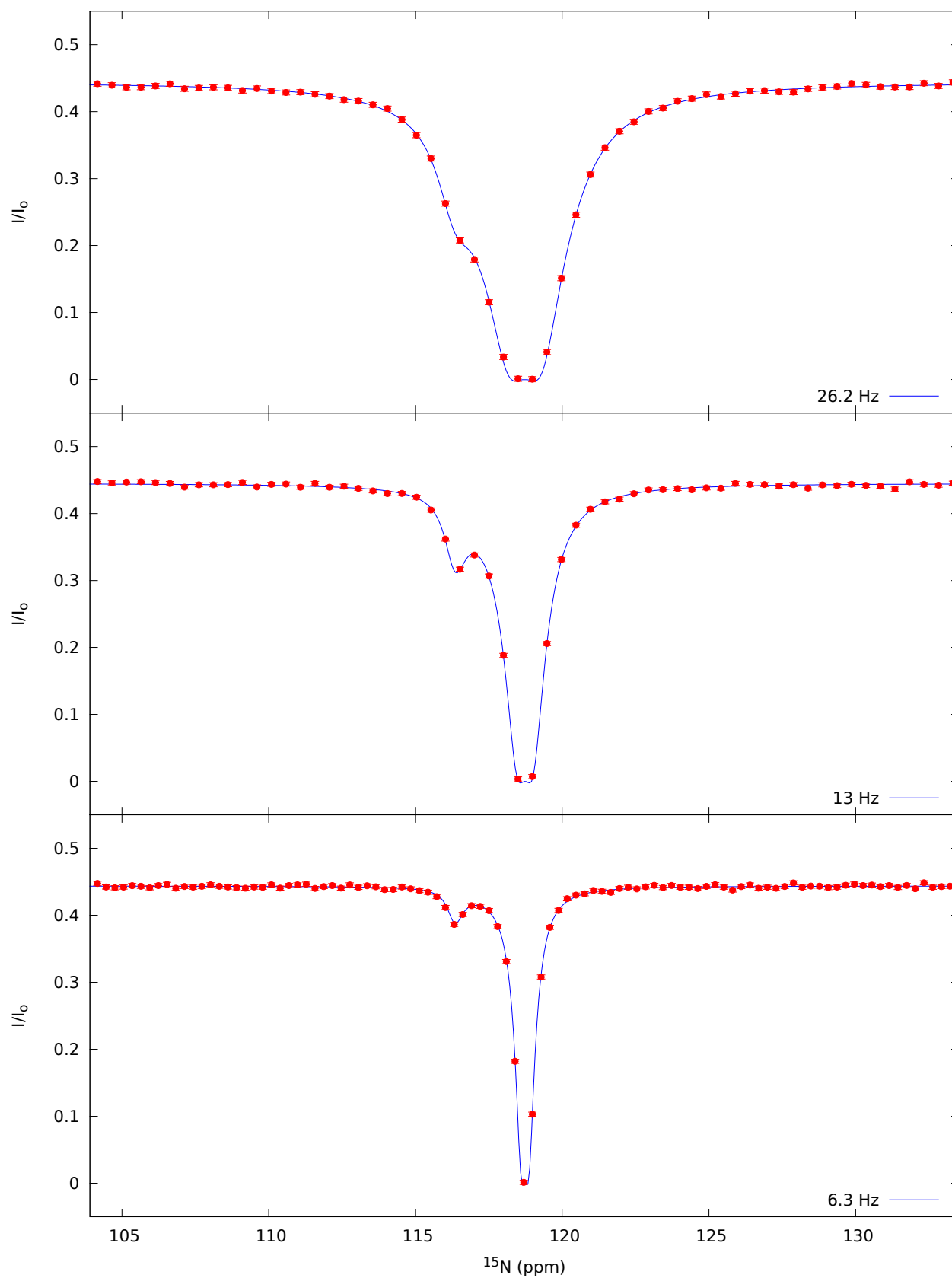
Residue 56



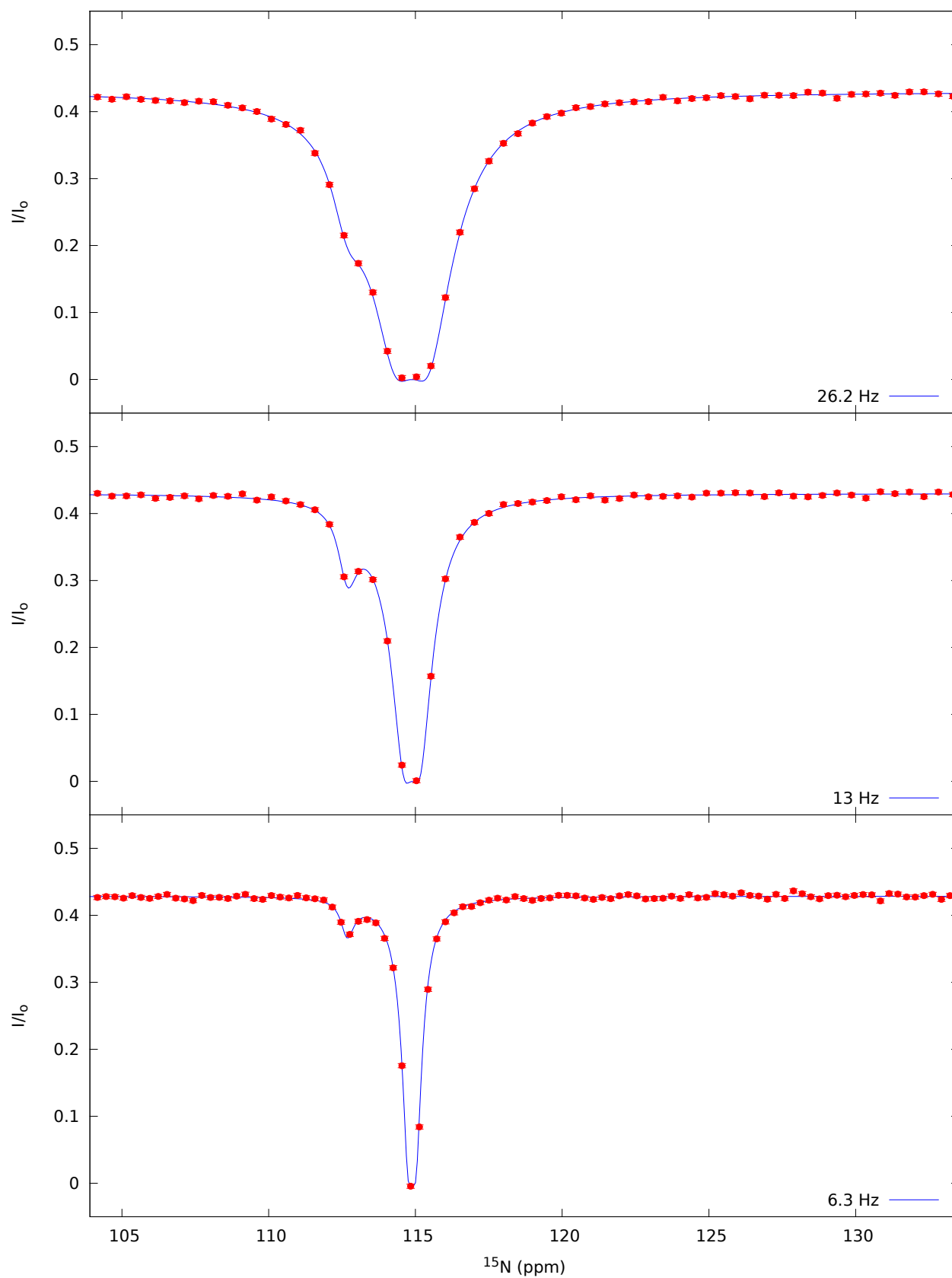
Residue 59



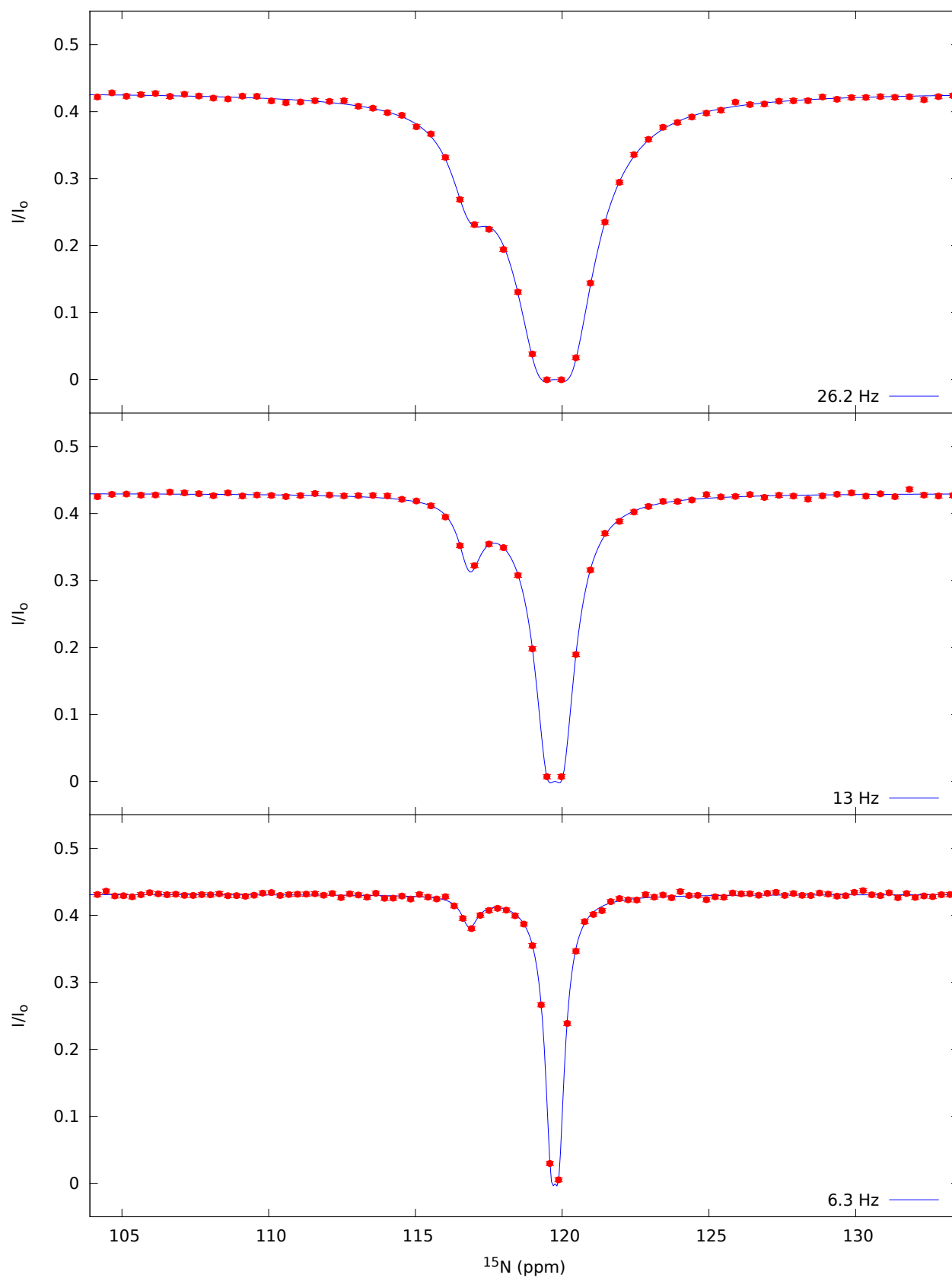
Residue 60



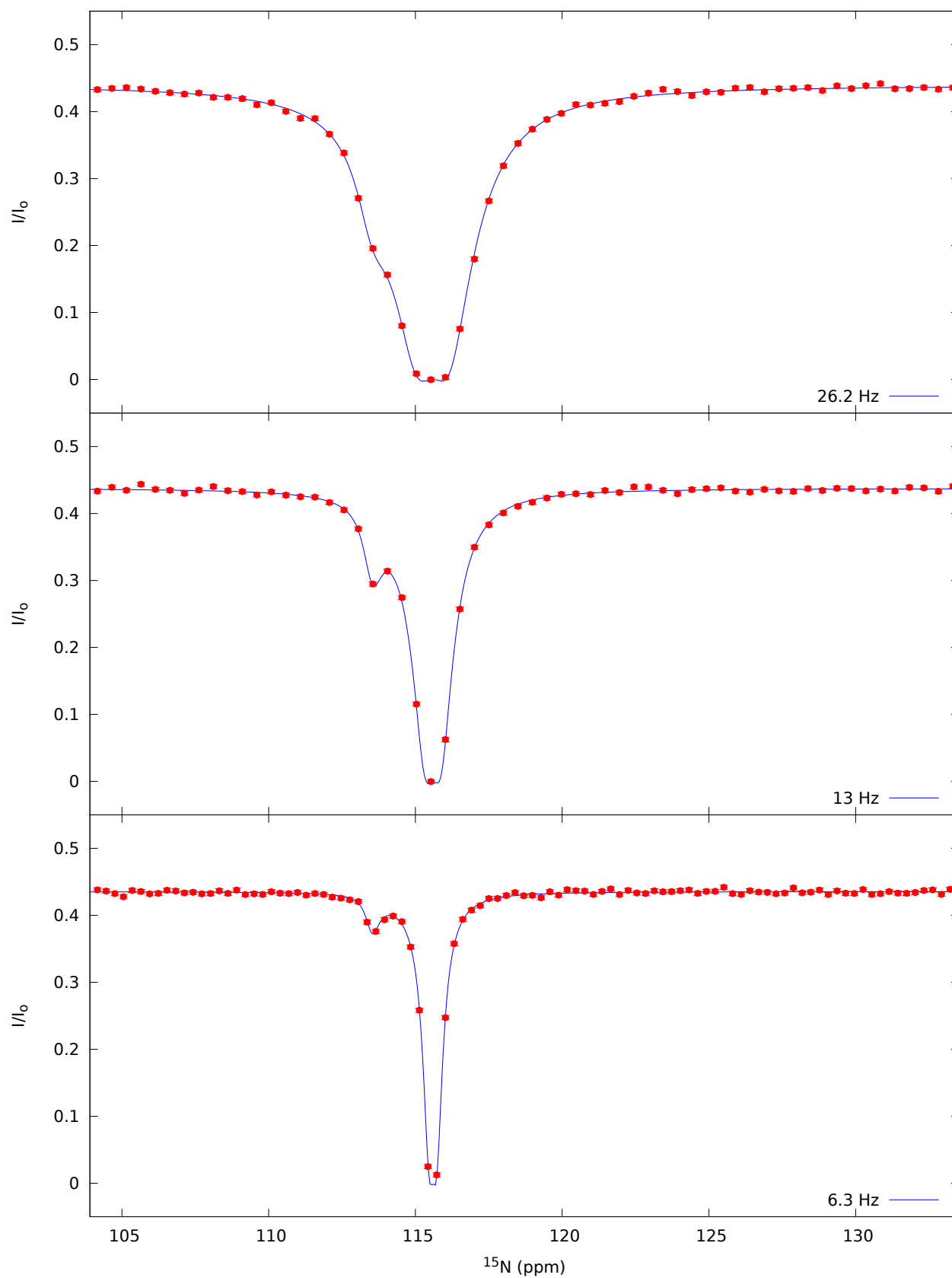
Residue 61



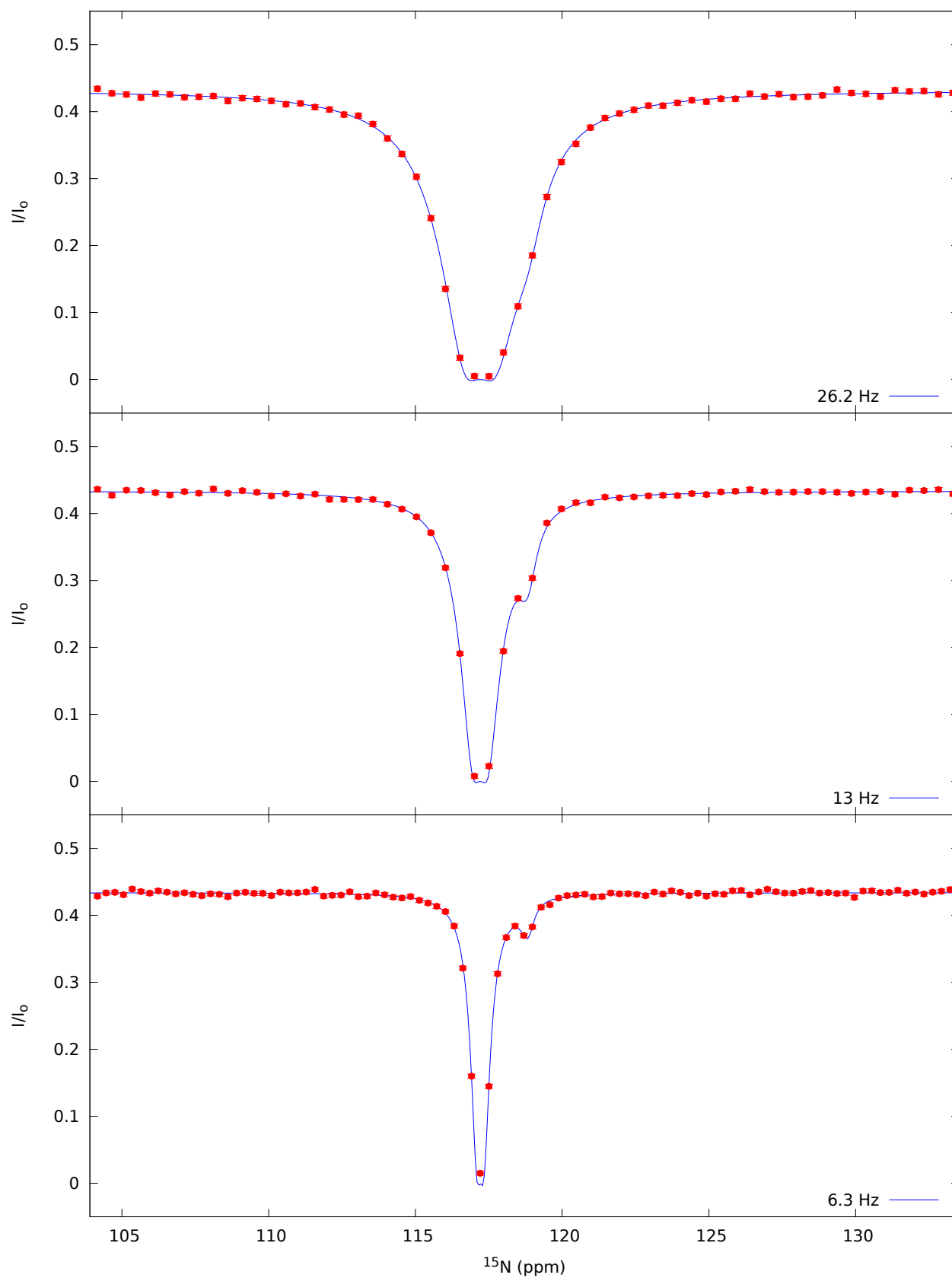
Residue 63



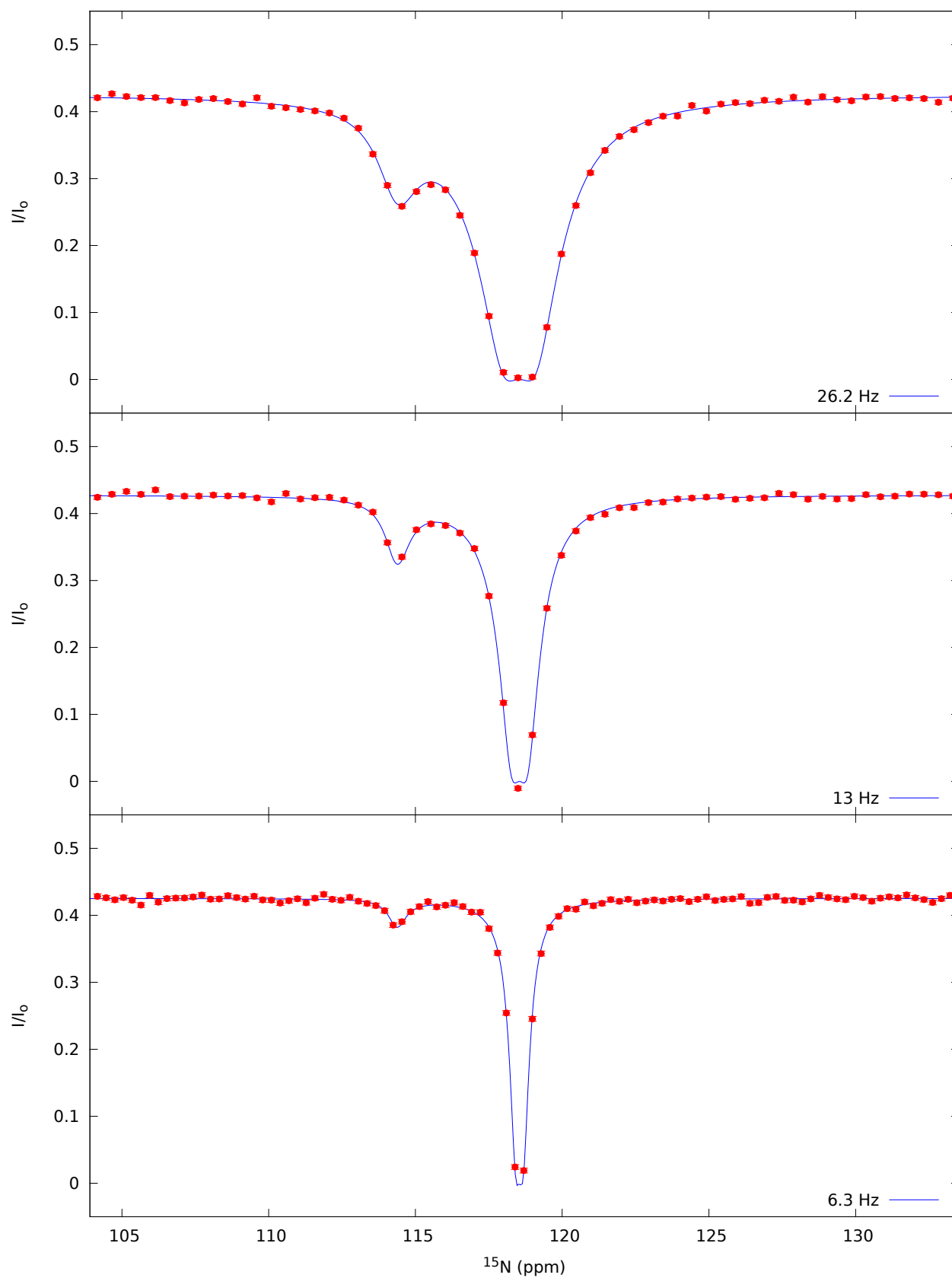
Residue 64



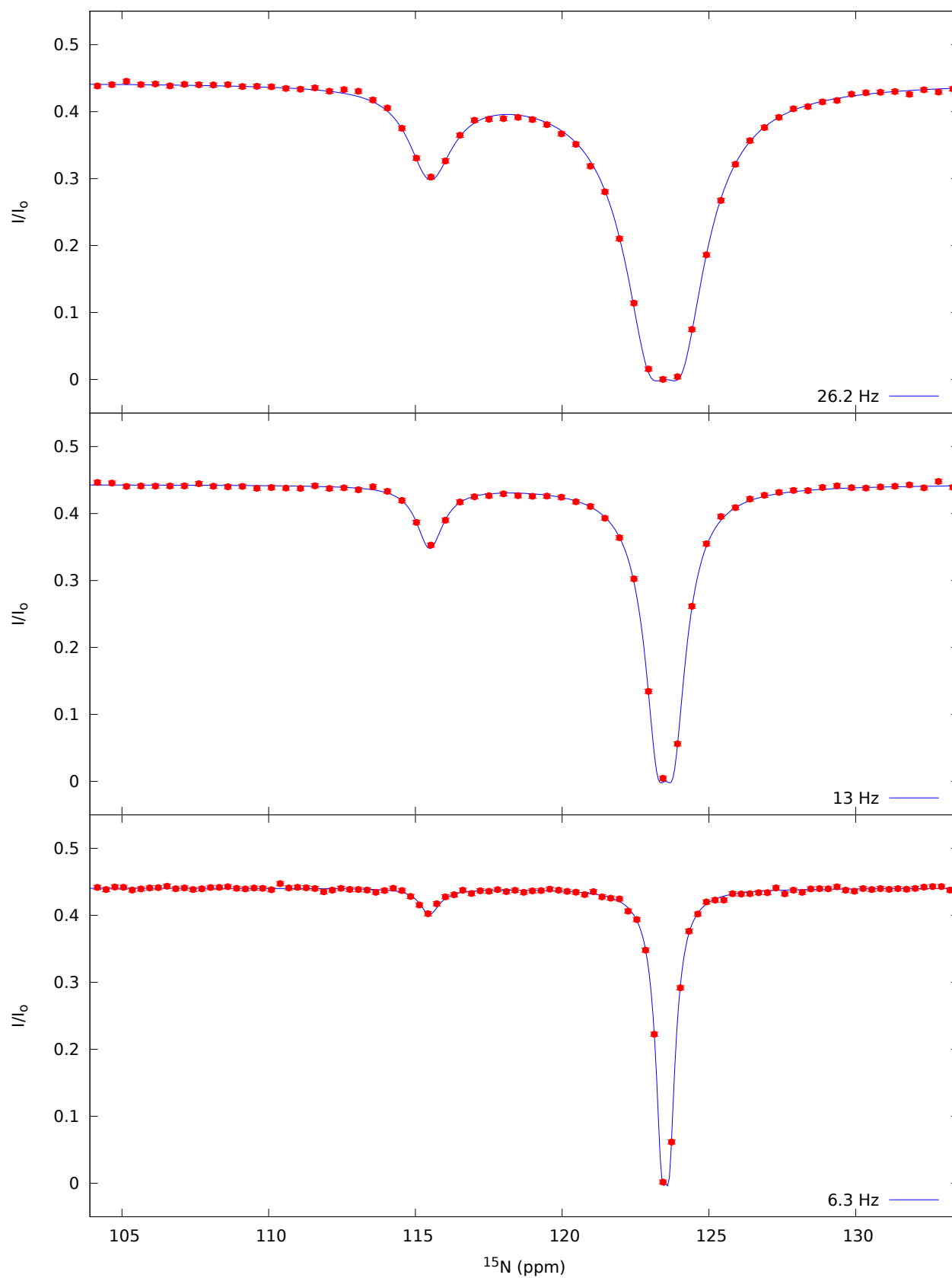
Residue 65



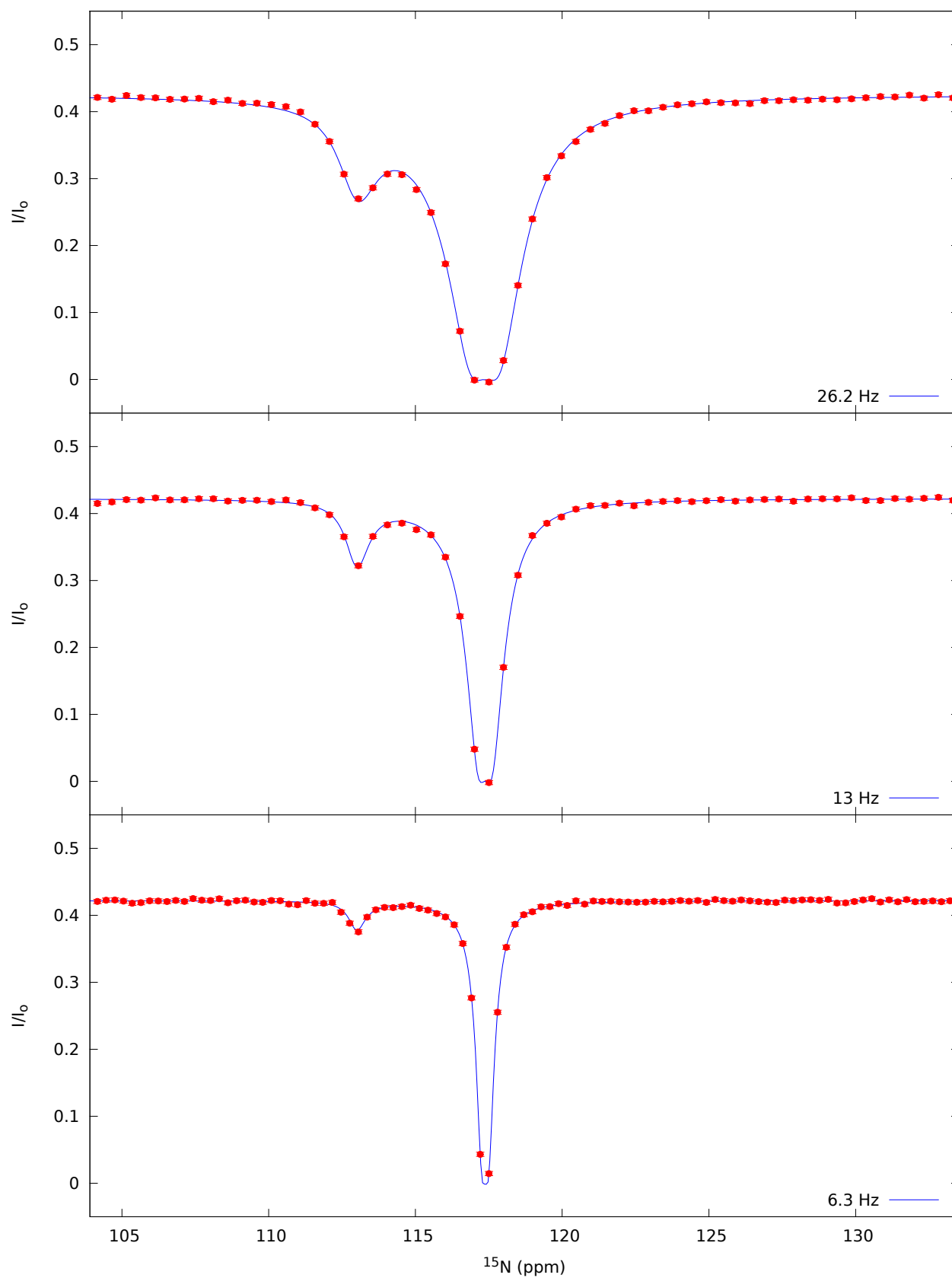
Residue 66



Residue 67



Residue 68



Residue 69

